**PASTEURELLA MULTOCIDAIN CAMELS: INCIDENCE,CAPSULAR AND VIRULENCE GENES CHARACTERIZATION**

1Ashraf A.Abd El Tawab, 1Fatma I.El -Hofy, 2Attia Al-Jeddawy and 2Ebtehal Abo-Hamdah

1Bacteriology, Immunologyand Mycology Department,Faculty of Veterinary Medicine Benha University . 2Animal Health Research Institute, Dokki – Giza**.**

**ABSTRACT**

*Pasteurella multocida* is the main cause of haemorrhagicsepticaemia in camels.This study looks at the isolation and molecular examination of haemorrhagicsepticaemia in camels from May 2014 to March 2016 in30 nasal swabs from MarsaMatruh and 120 lungs (70 lungs from camels slaughtered in Basateen abattoir in Giza Governorate and 50 from camels slaughtered in Al-Shohada abattoir at Al-Menofia Governorate) which were collected and subjected to clinical and postmortem examination as well as for bacteriological and molecular examination.*Pasteurellamultocida* was isolated from total samples at an incidance of 5(3.3% ).While the percentage of the isolation rate from 120 camel lungs was 5(4.2%) and from 30 nasal swabs was( 0%).In the pathogenicity test all *P.multocida* isolates were highly pathogenic . *P.multocida* isolates were identified by mPCR and23SR NA gene was amplified at 1432bp .Three out of total five isolates were identified as *P.multocida* type B with amplification at 760bp while the rest 2 isolates identified as *P.multocida* type A and amplified at 1044bp . Also PCR showdthat *toxA* gene was amplified in all isolates and giving product of 864bp but *ptfA* gene was not detected.It was concluded from this study that*P.multocida* in camels can be diagnosed with different methods such as confirmatory biochemical and molecular assays.

**Keywords:***Pasteurellamultocida*,Haemorrhagicsepticaemia, ,camel **,**Egypt

**1.INTRODUCTION**

*Pasteurellamultocida*, a member of family Pasteurellaceae is responsible for

haemorrhagic septicemia (HS) or pasteurellosis in camels all over the world (Mochabo et al.,2006,Harper et al.,2014).

*Pasteurellamultocida*is considered to be part of the normal respiratory flora of camels and other animals but become pathogenic and causes the disease when the resistance of the body of camel is lowered by harmful environmental influences such as sudden changes in weather,transportation over long distances, deficiencies of dietary nutrition and heavy parasitic infestation as trypanosomiasis (Ewer et al., 2004 and Walied 2006).

Morbidity is low, but mortality can be as high as 80%. Carrier or sick camels can be a source of infection to other animals specially in young calves. *P.multocida* causes a septicemia in camels within 10-24 hours, leading to high temperature, swelling in the throat region, pulmonary edema, fibrinous pneumonia, diarrhea and death usually occurs within 2-3 days (Walied 2006 ).

Identification of*P. multocida*depends upon the isolation and identification of the organism from suspected materials by cultural, morphological and biochemical characters. They are mostcommonly used(Townsend et al., 1998 and Hetal 2004) .

Also PCR used to accurate, rapid detection of toxigenicP. Multocida from swabs andtissue (Carol et al ., 1996).MultiplexPCRis analternative to comparative phenotypic tests for the capsular typing of P.multocida ,for the stimultaneous ,rapid detection of genes and provides a greater capacity for strain typing (Furian et al.,2014).

Therefore Pasteurellosis is considered to be important diseases in camels due to economic losses.The present work was planned to isolation and identification of *Pasteurella multocida* related from camels in Egypt .

**2.MATERIAL AND METHODS**

*2.1.Samples collection*

A total of 150 camels samples from Egypt were collected 30 nasal swabs from camels in MarsaMatruh(25 with respiratory infection and 5 apparently healthy animals) and 120 lung samples( 70 camel lungs from Basateen abattoir in Giza Governorate (60 with respiratory infection and 10 apparently healthy animals) and 50 camel lungs from Al-Shohada abattoir in Al-Menofia Governorate),all ofthis had respiratory infection. All of these samples were aseptically collected and transferred immediately in icebox to the laboratory.

*2.2.Bacteriological examination*

The samples were inoculated directly on Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hrs then streaked onto blood agar ,DAS medis and MacConkey agar plates. The suspected colonies which showd typical colonial appearance of *P.multocida* were identified by morphologically and biochemical methods .

*2.3.Biochemical examination*

The suspected colonies of *P.multocida* isolates were subjected to different biochemical tests such as catalase,oxidase,indole production and sugar fermentation test(Quinn et al.,2002) .AlsoAnalytical Profile Index 20 NE test(API 20 NE, biochemical rapid test, BioMerieux, France)was used.

*2.4. Pathogenicity test*

To determine pathogenicity of the identified *P.multocida* isolates by inoculation into BHI broth and incubated at 37°C,then(0.2ml) Inoculum of the isolates was injected intraperitonially into the Swiss Albino mice then identify and recorded the time of death of each mouse during the next 24hrs.

*2.5.Multiplex PCR assay*

PCR amplify-cation of 23S ribosomal DNA of *P.multocida* isolates was carried out using the following primers (table1).For identification of*P.multocida* isolates by mPCR the suspected colonies were cultured in BHI broth and incubated at37ºC for 24hours.

DNA was extracted as described inSambrook et al., (1989) and QIAamp DNA mini kit instructions) with minor modification. Only one ml of cultured colonies in BHI centrifuged then discarded the supernatant and washed the pellets and centrifuged again.After extraction of DNA of each bacterialisolates PCR master mix was prepared according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit mixingdeoxy-nucleoside .Amplified PCR products were run on 1.2% agarose gel by Agar gel electrophoresis(Sambrook et al., 1989) with minor modification and visualized by gel documentation system (Kodak) after staining byethidium bromide .

**3.Results**

*3.1.Clinical signs and P.m.Lesions*

A total of 150 samples from camels were collected(30 nasal swabs and 120 lungs). Clinicalydiseased camels showed signs of muco purulent nasal discharge, Pyrexia (fever might reach 40°C) in case of septicaemia ,salivation , lacrimation ,anorexia,occasionally diarrhea which sometimes contain blood.Also diseased camel died within9dayes.P.M.lesionswerepurulentbronchopneumonia,hydrothorax,emphysema,fibrinous pericarditis,red and gray hepatization,inflammation of thoracic lymph nods with heamorrhage ,adhesion of the lung to the thorax and general congestion in internal organs specialy pneumonic lung.

*3.2.Bacteriological and Biochemical identification of P.multocida isolates*

On blood agar and on DAS medium (1% crystal violet) after incubated anerobically at 37ºC for 24hrs all the isolates of *P.multocida* produced smooth or mucoid, non-haemolytic, round ,grayish colonies and accompanied by a characteristic "mousy" odour due to metabolic products. While the isolates of*P.multocida*failed to grow onMacConkey's agar.*P.multocida*showd as grame negative, non motilecoccobacillaryrods and a distinct bipolar staining reaction with Leishman’s stain.Also suspected positive cultural colonies suspected tobiochemicaland confirmatory EPI 20 NE tests andshowed identical biochemical reaction to *P.multocida*(Table2).

*3.3.Incidance of P.multocida in camels*

anincidance of isolation of P.multocida from total 150 samples was 5(3.3%).While the percentage of isolation of *P.multocida* from 120 lungs was 5(4.2%) and (0%) from 30 nasal swabs( table3).

*3.5.Pathogenicity test*

All 5*P.multocida*isolates(Biochemical identified) after inoculation in mice were highly pathogenicwhere all inoculated mice were dead within 24hrs with P.M.finding of septicemia and *P.multocida*was reisolated from heart blood and shown specific bipolarity of *P.multocida* organism by Leishman’s stain.

*3.6.Multiplex PCR assay*

In this study five isolates found to be positive for *P.multocida* with the same incidence obtained by EPI 20NE and giving amplified segment product of 1432 bp.

Also the results of capsular typing of *P.multocida* and detection of some virulent genes (*toxA,pdfA*)of *P.multocida*by mPCR were 3 isolates identified as *P.multocida* type B and amplified at 760bp while (2) isolates identified as *P.multocida*type A and amplified at 1044bp. Also*toxA*gene was amplified in all isolates and giving product of 864bp but *ptfA* gene was not detected in any *P.multocida* isolates (table4).