Pasteurella multocida in camels: incidence, capsular and virulence genes characterization

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ABSTRACT

Pasteurella multocida is the main cause of hemorrhagic septicemia in camels. This study deals with the isolation and molecular examination of hemorrhagic septicemia in camels from May 2014 to March 2016 from 30 camel nasal swabs in Marsa Matruh and 120 camel lungs (70 slaughtered in Basateen abattoir in Giza Governorate and 50 slaughtered in Al-Shohada abattoir at Al-Menofia Governorate). All collected samples were subjected to clinical, postmortem examination as well as for bacteriological and molecular examination. *Totally P. multocida* was isolated from the examined samples with percentage of 5(3.3%). While the percentage of the isolation rate from 120 camel lungs was 5(4.2%). In contrast, all 30 nasal swabs were negative. In the pathogenicity test, all P.*multocida* isolates were highly pathogenic. *Pasteurella multocida* isolates were identified by PCR and 23 S RNA gene was amplified at 1432bp. Three out of five isolates were identified as *P.multocida* type B with amplification at 760bp while other two isolates identified as *P.multocida* type A and amplified at 1044bp . Also, PCR showed that *toxA* gene was amplified in all isolates and giving product of 864bp but *ptfA* gene was not detected. As conclusion, *P.multocida* in camels can be diagnosed with different methods such as confirmatory biochemical and molecular assays.

Keywords: *Pasteurella multocida*, Hemorrhagic septicemia, camel, Egypt.

(http://www.bvmj.bu.edu.eg) **(BVMJ-31(2):171 -175, 2016)**

1. INTRODUCTION

Pasteurella multocida, a member of family Pasteurellaceae, is responsible for hemorrhagic septicemia (HS) or pasteurellosis in camels all over the world (Harper et al., 2014; Mochabo et al., 2005). *Pasteurella multocida* is considered to be a part of the normal respiratory flora of camels and other animals but it becomes pathogenic and causes the disease when the resistance of the camel body 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 (Ewers et al., 2004; Saber, 2006). Morbidity of pasteurellosis is low, but mortality may be high (80%). Carrier or sick camels consider as source of infection to other animals specially in young calves. *P.multocida* causes a septicemia in camels within 10-24 hours, leading to fever, swelling in the throat region, pulmonary edema, fibrinous pneumonia, diarrhea and death usually occurs within 2-3 days (Saber, 2006). Identification of *P. multocida* depends upon the isolation and identification of the organism from suspected materials by cultural, morphological and biochemical characters done (Patel, 2004; Townsend et al., 1998). Also, PCR is used to accurate, rapid detection of toxigenic *P. Multocida* from swabs and tissues (Carol et al., 1996). Multiplex PCR is an alternative to comparative phenotypic tests for the capsular typing of *P.multocida* , for simultaneous and rapid detection of genes that provides a greater capacity for strain typing (Furian et al., 2014). Therefore, Pasteurellosis is considered an important disease in camels due to its higher economic losses. The present work was planned to isolate and identify *Pasteurella multocida* from camels in Egypt.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 150 camel's samples were collected from 30 camel nasal swabs in Marsa Matruh (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), had history of 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 into Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours then streaked onto blood agar, DAS media and MacConkey agar plates. The suspected colonies which showed typical colonial appearance of *P.multocida* were identified by morphological 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. Also, Analytical Profile Index 20 NE test (API 20 NE, biochemical rapid test, BioMerieux, France) was done.

2.4. Pathogenicity test

Pathogenicity of the identified *P.multocida* isolates was determined 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 identified and recorded the time of death of each mouse during the next 24 hours.

2.5. Multiplex PCR assay

PCR amplify-cation of 23S ribosomal DNA of *P.multocida* isolates was carried out using the following primers (table1). DNA was extracted as described by Sambrook et al. (1989) using 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 bacterial isolates PCR master mix was prepared according to Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit mixing deoxy-nucleoside. Amplified PCR products were run on 1.2% agarose gel by Agar gel electrophoresis and visualized by gel documentation system (Kodak) after staining by ethidium bromide.

3. RESULTS

3.1. Clinical signs and P.M. Lesions on investigated camels

A total of 150 samples from camels were collected (30 nasal swabs and 120 lungs). Clinically diseased camels showed signs of mucopurulent nasal discharge, Pyrexia (fever might reach 40°C) in case of septicemia, salivation, lacrimation, anorexia, occasionally diarrhea which sometimes contain blood. Also, diseased camel died within 9 days. P.M. lesions were purulent bronchopneumonia, hydrothorax, emphysema, fibrinous pericarditis, red and gray hepatization, inflammation of thoracic lymph nodes with hemorrhage, adhesion of the lung to the thorax and general congestion in internal organs especially pneumonic lung.

3.2. Bacteriological and Biochemical identification of P.multocida isolates

On blood agar and DAS medium (1% crystal violet) after incubated anaerobically at 37ºC for 24hrs, all the isolates of *P.multocida* produced smooth or mucoid, non-hemolytic, round , grayish colonies and accompanied by a characteristic "mousy" odor due to metabolic products. While, the isolates of *P.multocida* failed to grow on MacConkey's agar. *P.multocida* showed as gram negative, non-motile coccobacillary rods and a distinct bipolar staining reaction with Leishman's stain. Also suspected positive cultural colonies was subjected to biochemical and confirmatory API 20 NE tests and showed identical biochemical reaction to *P.multocida* (Table 2).

3.3. Incidence of P.multocida in camels

An incidence 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 (table 3).

3.4. Pathogenicity test

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

3.5. Multiplex PCR assay

In this study five isolates found to be positive for *P.multocida* with the same percentage obtained by API 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 PCR 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.

Table I: Primers used for the detection of virulence-associated genes in isolates.

Target gene	Primers sequences	Amplified segment (bp)	Reference
23S Rrna	GGC TGG GAA GCC AAA TCA AAG CGA GGG ACT ACA ATT ACT GTA A	1432	(Miflin and Blackall, 2001) & (OIE Terrestrial, 2012)
Serogroup A	TGC-CAA-AAT-CGC-AGT-GAG TTG-CCA-TCA-TTG-TCA-GTG	1044	
Serogroup B	CAT-TTA-TCC-AAG-CTC-CAC-C GCC-CGA-GAG-TTT-CAA-TCC	760	
Serogroup E	TCC-GCA-GAA-AAT-TAT-TGA-CTC GCT-TGC-TGC-TTG-ATT-TTG-TC	511	
ToxA	CTTAGATGAGCGACAAGG GAATGCCACACCTCTATAG	864	(Tang et al., 2009)
PtfA	TGTGGAATTCAGCATTTTAGTGTGTC TCATGAATTCTTATGCGCAAAATCCT GCTGG	488	

Table (2) Results of biochemical identification of the isolated *Pasteurella multocida* using standard laboratory testes:

Result	Biochemical tests
+ve	Oxidase
+ve	Catalase
+ve	Indole production
-ve	Growth on MacConkey's'agar
-ve	Urease test
+ve	TSI
-ve	M.R .and V.P. tests
-ve	Hemolysis on blood agar
-ve	Citrate utilization
+ve	Nitrate reduction
+ve	Glucose
+ve	Mannitol
+ve	Sucrose
+ve	Mannose
-ve	Maltose
-ve	Arabinose
-ve	Lactose
-ve	Dulcitol
-ve	Salicin
-ve	Inositol
-ve	Trehalose

Table (3) The percentage of *Pasteurella multocida* isolated from Lungs and nasal swabs of one humbled camels from different geographical areas examined by biochemical examination and API 20 NE technique.

Type Of samples	Area	No. of examined samples	Results			
			No of +ve samples	%	No. of -ve samples	%
Nasal swabs	Mursa Matruh	30	0	0%	100	100%
Lungs	Bassatine abattoir	70	1		115	
	Al-Shohada abattoir	50	4			95.8%
	Total	120	5	4.2%		
Total number		150	5	3.3%	145	96.6%

Table (4): Results of molecular investigation of *P.multocida* isolates by multiplex PCR.

Sample	Kmt Result	Serotype A	Serotype B	Serotype E	toxA	ptfA
1	Not done	_	+	_	+	-
2	Not done	_	+	_	+	-
3	Not done	_	+	_	+	-
4	Not done	+	_	_	+	-
5	Not done	+	_	_	+	-

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).

4. DISCUSSION

Pasteurella multocida is the main cause of hemorrhagic septicemia in camels. In the present study, five isolates from total 150 samples (30 nasal swabs and 120 lungs) were found to be positive for *Pasteurella multocida* with an incidence of (3.3%). This results was nearly in coordinating with that reported by Abo-Elnaga and Wafaa (2012) who examined 175 lungs from camels slaughtered at Matrouh main abattoirs. They found that, the incidence rate of *P. multocida* was (2.9%. Also, Wareth et al. (2014) who examined a total of 500 lung tissues from apparently healthy camels which imported from Sudan and Slaughtered at El-Warrak slaughter house and they isolated Pasteurella spp. at an incidence of 2.85%.While such results disagree with Kibruyesfa (2015) who isolated Pasteurella spp. from 207 camel lungs from slaughtered camels at Addis Ababa Akaki Abattoirin an incidence of 5.7%.

In this study, the bacteriological and microscopical examination of *P.multocida* isolates showed short bacillary forms giving a distinct bipolar staining reaction with Leishman's stain. The suspected growing colonies were studied by morphological, confirmatory biochemical and API 20 NE examination and revealed five isolates identified as *P.multocida*. This result agreed with those of Seleim et al. (2003) and Jabeen et al. (2013).

The recorded results of pathogenicity of five isolated *pasteurella multocida* isolates in mice proved that all isolates were highly virulent and pathogenic to mice with a mean death time 24 hours with P.M.finding of septicemia. These results agreed with those of Townsend et al. (2000) and OIE Terrestrial (2008). Multiplex PCR was used for capsular typing and detection of some virulence genes (*toxA and ptfA*) of isolated *P. multocida*. The results of molecular examination were five *P. multocida* isolates in same percentage of which obtained by confirmatory API 20NE were identified and 23S RNA gene was amplified at 1432bp. Also 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. These results agreed with Tahamtan et al. (2016) who proved that capsular PCR assay identified *P.multocida* serotype A and B in camels by the specific primers for capsular types A and B amplifying 1044 and 760 bp respectively. So *P.multocida* type B was the main cause of hemorrhagic septicemia in camels. Also Sahragard et al. (2011) developed a multiplex PCR in the *P.multocida* isolates which more rapid and specific than biochemical analysis and mice bioassay and *toxA* gene was found in all *P.multocida* isolates.

5. CONCLUSION

Camel *Pasteurellosis* is considered to be important epidemiological and diagnostic diseases in camels due to economic losses amongst the camels and need more studies to management and control the disease in Egypt .

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