Saudi Journal of Biological Sciences (2016) **23**, 48–53

King Saud University

**Saudi Journal of Biological Sciences**

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

# Comparative studies for serodiagnosis of haemorrhagic septicaemia in cattle sera

**Jakeen K. El-Jakee [a], Samah Said Ali [b], Soumaya Ahmed El-Shafii [b], Ashgan M. Hessain [a,c], Abdullah A. Al-Arfaj [d], Moussa I. Mohamed [a,d,*]**

[a] *Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, P. O. Box 2446, Cairo 14242, Giza, Egypt*
[b] *Bacteriology Department, Animal Health Research Institute, P. O. Box 2440, Giza, Dokki, Egypt*
[c] *Department of Health Science, College of Applied Studies and Community Service, King Saud University, P. O. Box 22459, Riyadh 11495, Saudi Arabia*
[d] *Department of Botany and Microbiology, College of Science, King Saud University, P. O. Box 2455, Riyadh, Saudi Arabia*

Received 16 April 2015; revised 1 June 2015; accepted 10 June 2015
Available online 16 June 2015

**KEYWORDS**

Haemorrhagic septicaemia;
Serodiagnosis;
HAT;
IHAT;
ELISA

**Abstract** Haemorrhagic septicaemia caused by *Pasteurella multocida* is a major epizootic disease in cattle and buffaloes in developing countries with high morbidity and mortality rate. In the present study, a total of 88 *P. multocida* isolates were isolated from 256 nasopharyngeal swabs and lung tissues samples (34.4%) during the period from January, 2013 to March, 2014 from different governorates located in Egypt. Dead calves showed the highest percentage of *P. multocida* isolation followed by the emergency slaughtered calves, diseased calves then apparently healthy ones. These isolates were confirmed as *P. multocida* microscopically, biochemically by traditional tests and by API 20E commercial kit then by PCR. The percentages of positive serum samples using somatic antigen and micro-agglutination test at 1/1280 diluted serum were 10%, 54.49% and 0% in apparently healthy, diseased and emergency slaughtered samples, respectively whereas, the percentages using capsular antigen and indirect haemagglutination test were 40%, 60.89% and 60% in apparently healthy, diseased and emergency slaughtered samples, respectively. The ELISA showed the highest sensitivity for diagnosing *P. multocida* in apparently healthy, diseased and emergency

* Corresponding author at: Department of Botany and Microbiology, College of Science, King Saud University, P. O. Box 2455, Riyadh, Saudi Arabia. Tel.: +966 56749553; fax: +966 14678456.
E-mail addresses: iha319@yahoo.com (J.K. El-Jakee), ahessan@ksu.edu.sa (A.M. Hessain), imoussa1@ksu.edu.sa (M.I. Mohamed).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.06.011

slaughtered animals with percentages of 42%; 92.9% and 80%, respectively. The obtained results revealed that the ELISA using capsular antigen of *P. multocida* is a more sensitive and specific serological test for diagnosis of haemorrhagic septicaemia.



## 1. Introduction

*Pasteurella multocida* causes a serious disease in a wide range of avian, animal species and humans (Christensen and Bisgaard, 2006; Descamps et al., 2012) causing high economic losses in both developed and developing countries (Dziva et al., 2008). Haemorrhagic septicaemia (HS) is a major epizootic disease in cattle and buffaloes in many countries with high morbidity and mortality. Outbreak of HS among buffaloes and cattle could be due to the consumption of river water contaminated with infected carcasses with *P. multocida* beside aerosol route (Jesse et al., 2013). Several serological tests are used for the identification of the HS including slide agglutination test (Namioka and Murata, 1961), indirect haemagglutination test for capsular typing (Carter, 1955), the agar gel immunodiffusion test (Heddleston et al., 1972).

ELISA was a sensitive and specific test. The boiled-cell extract antigen was chosen as the best antigen prepared to be used in ELISA than whole bacterium antigen which is the least sensitive while, lipopolysaccharide antigen was more difficult to prepare and may be type specific (Klaassen et al., 1985). The aim of the present study was to evaluate the different serological tests in the diagnosis of haemorrhagic septicaemia.

## 2. Materials and methods

### 2.1. Samples

Under aseptic conditions, 206 nasopharyngeal swabs were collected from apparently healthy calves ($n = 50$) and from diseased calves suffering from septicemia and respiratory manifestations ($n = 156$). Also 50 lung samples collected from emergency slaughtered calves ($n = 35$) and dead calves ($n = 15$) were obtained from pneumonic calves during the period from January, 2013 to March, 2014 from different governorates located in Egypt. For serodiagnosis, a total of 221 blood samples were collected from apparently healthy animals ($n = 50$), diseased calves ($n = 156$) and emergency slaughtered animals ($n = 15$). All animals were chosen from different private farms in El-Behera and Kafer El-Sheikh governorates, Egypt which did not vaccinate animals against haemorrhagic septicaemia. The collected samples were transferred as soon as possible to the laboratory for bacteriological examination. Sera were separated from the blood samples for serodiagnosis of *P. multocida*.

### 2.2. Isolation and identification of P. multocida

The collected samples (nasopharyngeal swabs and lung tissues) were inoculated in Casein/sucrose/yeast broth (Oxoid) for 6–8 h. A loopful was cultivated on Casein/sucrose/yeast agar (CSY), blood agar and MacConkey agar plates then incubated at 37 °C for 48 h. The suspected colonies with culture characters of *P. multocida* were identified traditionally according to Quinn et al. (2002) using API 20 E tests (BioMérieu).

### 2.3. Polymerase chain reaction (PCR)

The procedure of PCR analysis was performed according to Townsend et al. (1998) and the instructor manual provided with kits (Qiagen) with some modification. A PCR reaction mixture (20 µl total volume) was prepared as follows: 3 µl nuclease free water, 10 µl Hot Start Taq plus master mix (2×), 2 µl Coral Load concentrate (10× optional), 1 µl forward species specific primer KMT1T7 (5′-ATCCGCTATTTACCC AGTGG-3′), 1 µl reverse primer KMT1SP6 (5′-GCTGTAA ACGAACTCGCCAC-3′) and 3 µl Extracted DNA. The PCR cycling profile consisted of one cycle for initial heat activation of 95 °C for 5 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min and final cycle for 10 min at 72 °C. Then, the PCR products were visualized by agarose gel electrophoresis. Agarose gel electrophoresis was carried out according to Sambrook et al. (1989) to evaluate the amplified fragments using standard PCR markers and 100 bp ladder.

### 2.4. Pathogenicity test in mice (OIE, 2008)

Each isolate was inoculated in 3 mice intramuscularly by 0.1 ml of 24 h broth culture of *P. multocida* isolate. The turbidity of the broth was adjusted to McFarland's tube no 4. The mice were kept under observation for 72 h after inoculation, dead mice were necropsied and live mice were killed after 3 days and necropsied. Blood films were prepared and stained with Giemsa stain and examined for the presence of bipolarity.

### 2.5. Preparation of hyperimmune serum and P. multocida antigens

Hyperimmune serum was prepared in New-Zealand rabbits (2–3 kg) according to OIE, 2008 to be used as positive control in the serological assays. The somatic antigen from *P. multocida* strain was prepared in order to be used for micro agglutination test (MAT) and ELISA assays according to Namioka and Murata (1961). The capsular antigen from *P. multocida* strain was prepared in order to be used for indirect haemagglutination test (IHAT) and ELISA assays according to OIE, 2008.

### 2.6. Serodiagnosis assays

Micro agglutination test was applied in U-shape bottom plate using the prepared somatic antigen according to Shewen and Wilkie (1982). 1% sensitized sheep RBCs were prepared according to Carter (1955). Indirect haemagglutination test