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**RESEARCH ARTICLE**

# ISOLATION OF LEPTOSPIRES FROM BOVINE URINE AT MIDDLE PROVINCES IN IRAQ

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## *Abstract*

The present study was designed to investigate of Leptospirosis in cattle in middle of Iraq and isolate the Leptospires. One hundred and fifty urine samples were randomly collected from cattle at a slaughterhouse in the state of Karbala, Najaf and Babylon in Iraq. Direct examination using dark field microscopy (DFM) was carried out immediately after collection. Then samples were cultured in EMJH medium, and the culture identifying by staining, biochemical tests (catalase and oxidase) and examination by DFM . Results show eight samples were positive in DFM (5.3%) and thirteen samples positive by culturing (8.6%). In this study we concluded that Cattle can represent an increasing risk for severe leptospirosis in large population and urine easily can be used for diagnose leptospirosis.



## INTRODUCTION

Leptospirosis is an acute febrile and septicaemic disease caused by spirochaetes of the family *Leptospiraceae*, which includes saprophytic and pathogenic bacteria. Pathogenic leptospires are important in public health because they cause zoonotic disease **(Hernández- Rodríguez *et al*. 2011; Levett 2001; McBride *et al*. 2005)**. A wide range of host species, including humans, wildlife including rodents and carnivores, and domestic animals, act as reservoirs for *Leptospira* **(Jorge *et al*. 2011; Liu *et al*. 2006)**. Humans may be affected after direct contact with infected urine or more often indirectly via exposure to water or soil contaminated by the urine of infected animals **(Tansuphasiri *et al*. 2006; Vijayachari et al., 2008)**. Bovine leptospirosis is associated with abortion, lowered fertility, and decreased milk production in cattle, causing substantial economic losses **(Faine et al. 2000)**. Under tropical conditions, the disease is common. It is endemic in some tropical regions **(Lilenbaum and Souza 2003)**.

## MATERIAL AND METHODS

### Collection of samples

From April 2014 to September 2014 urine samples were collected from apparently healthy cattle, local breed, 1-5 years old, which were randomly selected during slaughtering in the state of Karbala, Najaf and Babylon.
Urine samples were obtained approximately ten minutes after slaughter. Approximately 5 ml were collected from each animal by direct bladder puncture. After collection, urine samples were transferred to the laboratory of the College of Veterinary Medicine, Baghdad University where they were examined by DFM, Optika® - Model B-353DK microscope, with 40X magnifications. The samples were subjected to the processing of culturing.

### Culture media

Ellinghausen McCullough Johnson Harris (EMJH) medium was prepared manually. The EMJH medium was prepared from basal medium and Albumin fatty acid supplement according to **Baron and Finegold (1990).** Broth, semisolid and solid medium prepared according to **Zimbro et al. (2009)** and 500 mg of 5-fluorouracil was added to 1000 ml of medium and 50 mg of Rifampicine also added **( Miraglia et al., 2009)**.

**Leptospira isolation**

0.5 ml of urine samples were inoculated into 5 ml of EMJH broth medium. The cultures were incubated at 29˚C and examine weekly up to 16 weeks by dark field microscopy (DFM) **(Ellis et al 1982)**. The turbidity of the medium gives an indication for growth of bacteria. Tubes that show this appearance subjected to direct examination by DFM. All the tubes subjected to the culturing in semisolid medium. The Dinger ring appearance was monitored as it is the characteristic feature for leptospiral growth in this medium. The tubes which show this appearance subjected to culturing in solid medium.

## RESULT AND DISSCUSSION

The result of the examination by DFM showed that 8 cases of 150 (5.3 %) urine samples were positive (Fig 1). The smear showed typical shape and typical movement of leptospires (Fig 2). This low percentage attributed to the possibility of the low number of the microorganisms in the field. Direct visualization of leptospires in fresh specimens is very difficult and cannot be considered as a reliable method for detecting carrier **(Lilenbaum et al, 2009).** The sensitivity of the method is low **(Magajevski et al., 2005; Zuerner, 2005)**.

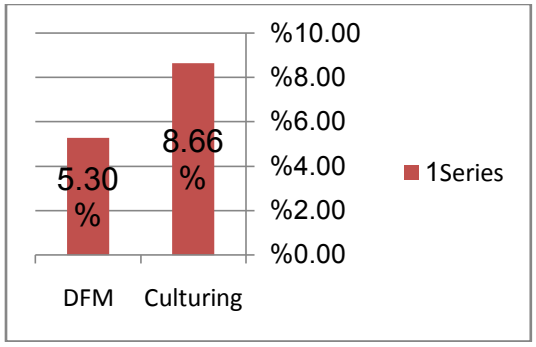


Fig 1 shows percentage of positive samples by DFM and Culturing

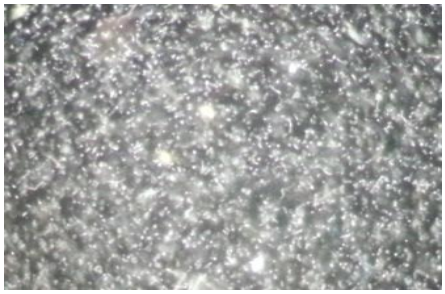

Fig 2 Leptospires under DFM magnification 40X

In isolation technique 13 isolates (8.6%) were identified as Leptospires. This identification was made by Microscopic examination and visualized the morphology of the bacteria when used Fontana and Congo red stains. The characteristic feature of motility of the bacteria was shown by DFM. **de Freitas et al., 2004** exhibit that **Alder et al. (1986)** and **Bolin et al. (1989)** recognize the difficulty in isolating leptospires, despite the presence of Leptospires in samples. **Moreira (1994)**, using 420 urine samples from bovine with leptospirosis, two isolates were obtained. The isolation technique is fastidious, and difficult for many reasons. **Zacarias et al. (2008)** stated that growth of leptospires observed after 12 weeks of inoculation as leptospires grow very slowly with generation time 16-18 hrs in log phase. Biochemical test showed characteristic result for leptospires as all the isolates give positive result in oxidase and catalase tests.

Dinger ring was shown in semisolid media and this is one of the characteristic features of leptospiral growth in this media (Fig 3). In solid media the colony was small and white grow subsurface and this in agreement with **Wuthickanun et al. (2013)**.

Fig 3 Semisolid media shows Dinger ring appearence

In study conducted in Mosul the isolation were 100% from 11 samples as they were collected from suspected cases of Leptospirosis **(Ajaj, 2013)** while **de Freitas et al. (2004)** conducted a study when he collected 3 samples of urine from three bovines classified as positive for leptospirosis by the direct examination under DFM, two samples were positive in culture. The high percentage of isolation in these two studies was attributed to the methods of collection as they collect the samples from suspected cases of Leptospirosis.

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