Bacteriological examination of bovine kidneys for leptospires in Plateau State, Nigeria

A.O. EZEH *, W.A. ELLIS **, E. KMETY ***,
A.A. ADESIYUN **** and P.B. ADDO ****

Summary: A total of 525 bovine kidneys obtained from Jos municipal abattoir were culturally examined for the presence of pathogenic leptospires. Six leptospiral strains belonging to two different serogroups were isolated. Five of the isolates were serologically homologous and belonged to a new serovar in the Pyrogenes serogroup. The remaining isolate was identified as serovar hardjo (genotype Hardjo-bovis).


INTRODUCTION

Leptospirosis is a major zoonosis which affects all domestic animals, wildlife and man. Bovine leptospirosis causes considerable economic losses from abortion, stillbirth, decreased milk production and infertility (1). Although serological tests are useful for the diagnosis of leptospirosis (10), they are not satisfactory for determining the identity of the infecting serovar. Cultural isolation provides an indication of the infection rate in a cattle herd, but it is difficult to do and is often unsuccessful (12). Infection of susceptible cattle may result in localisation of leptospires in the kidneys, with intermittent excretion in urine (6). Infected bovine urine is therefore the major source of leptospirosis to other cattle because of the volume of urine excreted and the duration of leptospirosis. Since pathogenic leptospires localise in the kidney, they are frequently isolated from the urine of live animals or from kidney tissue of slaughtered animals. However, urine is usually contaminated with other micro-organisms which interfere with the isolation of leptospires (11, 13). Previous serological studies have shown a high percentage of leptospirosis in man and livestock in different parts of Nigeria (3, 7, 14), but there have been few bacteriological isolations. In a bacteriological survey of leptospirosis in Zaria, Nigeria, leptospires were recovered from 5 of 74 bovine kidneys (4).

The aim of the present study was to attempt isolation of pathogenic leptospires from kidneys of slaughtered cattle in Plateau State of Nigeria.

* Leptospirosis Section, Bacteriology Division, National Veterinary Research Institute, Vom-Jos, Plateau State, Nigeria.
** Veterinary Research Laboratories, Stormont, Northern Ireland.
*** Leptospirosis Reference Laboratory, Bratislava, Czechoslovakia.
**** Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.
MATERIALS AND METHODS

Approximately one gram of kidney tissue, comprising the cortex and the medulla, was aseptically collected from slaughtered cattle at the Jos municipal abattoir. The kidney samples were macerated before they were placed into a 10 ml syringe and transferred to 10 ml liquid bovine serum albumin (BSA). The homogenate was allowed to stand on the bench for ten minutes before three serial dilutions of 1:50, 1:500 and 1:5000 were made with liquid BSA. From each dilution, 0.1 ml was inoculated into three 10 ml tubes of semi-solid modified Ellinghausen and McCullough (EMJH) medium (10) containing 150 µg/ml of 5-fluorouracil (Hoffman-La Roche Inc., NJ, USA) and 2% sterile rabbit serum. All the tubes were incubated at 30° C for 12 weeks (9) and examined weekly by dark-field microscopy for characteristic leptospiral growth. Suspect tubes were subcultured into fresh enriched EMJH medium and re-incubated at 30° C. Grossly contaminated cultures were discarded, as were tubes showing no evidence of leptospires after the 12-week period. When the subcultured leptospires had grown sufficiently, they were further purified by filtering through 0.22 µm millipore (8) into fresh semi-solid EMJH medium and re-incubated. The isolates were adapted to liquid EMJH medium by several subcultures until growth was sufficient for use as antigen. Duplicate pure cultures in semi-solid EMJH were sent to the Leptospirosis Reference Laboratory, Bratislava, Czechoslovakia and also to the Leptospirosis Research Laboratory, Belfast, Northern Ireland, for identification and confirmation of the isolates.

RESULTS

Six leptospiral strains belonging to two serogroups were isolated from 525 bovine kidneys (1.1% of which were positive) obtained from Jos municipal abattoir. Five serologically homologous isolates were serotyped as a new serovar in the Pyrogenes serogroup, and the sixth isolate was identified as serovar hardjo. Their identity was confirmed by cross-absorption tests, factor analysis and restriction endonuclease analysis (courtesy of Prof. E. Kmety of the Leptospirosis Reference Laboratory, Bratislava and Dr W.A. Ellis of the Leptospirosis Research Laboratory, Belfast).

DISCUSSION

Serovar hardjo (genotype Hardjo-bovis) has been isolated from bovine kidneys, apparently for the first time in Nigeria. The isolation of a new serovar of the Pyrogenes serogroup clearly indicates that extensive national surveys of wildlife and domestic animals will be needed to identify the prevalent leptospiral serovars in Nigeria. The isolation of hardjo from cattle is significant because this serovar is endemic in cattle populations of many countries (1, 5). Serovar pyrogenes has been isolated before from bovine kidneys in Nigeria (3), but the isolation of a new serovar of the Pyrogenes serogroup appears to be the second such report in Africa. Serovar pyrogenes has been isolated from water buffaloes in South-East Asia and Peru (4). The implications of the close relation of the five strains of Pyrogenes serogroup isolated in different sampling periods (January 1984 to February 1985) is unknown. Perhaps the
slaughtered cattle originated from the same ecological zone of Plateau State, since only a small number of serovars are usually endemic in any particular region or country.

It is concluded from the present study that leptospires are present in Nigerian cattle, despite the paucity of reports of clinical leptospirosis. Attempts to isolate further indigenous serovars in Nigeria should be intensified so that a rational control policy can be formulated.

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Résumé : Des reins de bovins obtenus à l'abattoir municipal de Jos, au nombre de 525, ont été mis en culture et la présence de leptospires pathogènes y a été recherchée. Six souches de leptospires, appartenant à deux sérogroupes différents, ont été isolées. Cinq d'entre elles étaient sérologiquement homologues et appartenaient à un nouveau sérovar du sérogroupe Pyrogenes. La dernière a été identifiée comme étant du sérovar hardjo (génotype Hardjo-bovis).


Resumen: Quinientos veinticinco riñones de bovinos obtenidos en el matadero municipal de Jos se pusieron en cultivo y se investigó en ellos la presencia de leptospiras patógenas. Se aislaron seis cepas de leptospiras, pertenecientes a dos serogrupos distintos. Cinco de ellas eran serológicamente homólogas y pertenecían a una nueva serovariante del serogrupo Pyrogenes. La última fue identificada como perteneciente a la serovariante hardjo (genotipo Hardjo-bovis).

PALABRAS CLAVE: Enfermedades de los bovinos - Investigación en mataderos - Leptospira interrogans - Nigeria - Serovariantes.
REFERENCES