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A COMPARATIVE EVALUATION OF THE ULTRASTRUCTURE AND PROTEIN YIELDS OF FOUR LEPTOSPIRES USING THE DETERGENT SOLUBILIZATION AND MECHANICAL DISRUPTION TECHNIQUES

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SUMMARY

The morphology of whole and modified leptospires and their protein yields were compared. The four leptospire serovars considered were *L. grippotyphosa*, *L. hamptoni*, *L. hardjo* and *L. pomona*. The ultrastructure of the mechanically disrupted leptospire showed that the outer envelope of the leptospire were released, although the process was not as efficient as the detergent solubilized technique. Additionally some axial filaments were also released. The protein yield of *L. hardjo* from the two techniques of leptospire protein preparation was significantly lower than the other test serovars ($P<0.01$). Overall, the protein yield of antigens produced by the SDS solubilization technique was significantly higher than the mechanical disruption technique ($P< 0.01$)

KEY WORDS: Leptospires, antigens, ultrastructure, protein yield.

INTRODUCTION

Leptospira are helical bacteria made up of four major parts; the outer membrane, axial filaments, periplasmic cylinder and the nuclear zone (Johnson and Faine, 1984). Auran *et al.* (1972) have shown that the outer membrane induces better immunity than whole cells. Since that work, several techniques have been advanced to produce leptospiral antigens for immunodiagnosis and immunoprotection. They include sonicated antigens (Adler *et al.*, 1980, Adler *et al*, 1981, Chappel *et al.*, 1992) heated antigens (Auran *et al.*, 1972, Terpstra *et al.*, 1987, Raoult *et al.*, 1989) and the mechanically disrupted antigen (Trueba *et al.*, 1990). In a comparative study of the antigens produced by various techniques, Trueba *et al* (1990) found variations in the sensitivities of the various antigens in their enzyme immunoassay work.

At present, there are few reports on the protein contents of various leptospiral antigens. Zeigler and Van Eseltine (1975) found that the SDS-solubilized outer

envelop of *L. pomona* contained 47 percent protein as well as some carbohydrate and lipid components.

The objectives of this work were to determine the ultrastructure of detergent and mechanical solubilized leptospires and assess the protein yield from the four leptospires studied.

MATERIALS AND METHODS

Leptospire strains and cultivation

L. grippotyphosa (Moska V), *L. hamptoni* (Hampton), *L. hardjo* (Hardjo-prajitno), and *L. pomona* (Pomona) were used for this work. These were grown in Ellinghausen and McCoullough media modified by Johnson and Harris, (1967) (EMJH). The National Leptospirosis Reference Centre, Ames, Iowa, supplied the leptospires.

Preparation of SDS - soluble and mechanically disrupted antigens

The four test serovars were grown in EMJH media for six days. The SDS soluble antigen was prepared as earlier described (Staak *et al.*, 1990, Agunloye *et al.*, 2001). The precipitate was resuspended in 5ml-distilled water and stored at -20^0C until needed.

The mechanically disrupted antigen was prepared using two liters of 6 day-old leptospires as previously described (Trueba *et al.*, 1990). The pellet protein fraction was suspended in 3ml 0.05M carbonate-bicarbonate buffer (pH 9.6). The protein content was determined as described by Bradford (1976).

Electron Microscopy

Whole, mechanically disrupted and SDS-solubilized antigens were studied morphologically using Electron microscopy. Pure preparations of leptospires and their fractions were resuspended in PBS and submitted to the Electron Microscopy Laboratory of the University of Georgia Veterinary School for ultrastructural study using a Joel® JM 1210 Electron Microscope.

RESULTS

Morphology and ultrastructure of the SDS -solubilized and mechanically disrupted leptospires

Plate 1 shows the morphology of the intact leptospire with slight mechanical disruption. The helical structure of the leptospire is seen. Over 31 coils are present. The salt altered leptospire with the cylindrical form is seen in Plate 2. Plate 3 shows the mechanically disrupted leptospire. However some discontinuous, broken, three-layered outer membrane is still present covering the protoplasmic cylinder. A cellular debris consisting of axial filaments and broken outer envelope is seen.

Protein Assay results of the leptospire antigens

The protein yield of the four test serovars after detergent solubilization and mechanical disruption was determined and the summary is presented in Table 1. The protein yield of *L. pomona* appeared highest, but this yield was not significantly higher than that of *L. grippotyphosa* ($P > 0.01$) by both techniques. However, the

yield of *L. hardjo* was significantly lower than the other test serovars. Also, the overall protein yield by the SDS solubilization technique was significantly higher ($P< 0.01$) than the mechanically disrupted antigens.

DISCUSSION

In this work, leptospiral outer membranes were released by the two methods of antigen preparation. SDS solubilization released the outer envelope. This ability to solubilized leptospiral outer membrane has earlier been demonstrated (Auran *et al.*, 1975, Fathalla and Coglan, 1980, Brown *et al.*, 1991).

The outer membrane of the leptospire is its outermost part, which is said to have a trilaminar structure ((Nauman *et al.*, 1969, Hovind-Hougen, 1986) although five-layered structures have been reported in *L. pomona* (Zeigler and Van Eseltine, 1975). Similarly, in their electron microscopy study of *L. patoc* and *L. canicola*, Anderson and Johnson (1968) reported five-layered outer envelopes. These workers described the outer membrane as three layers interspersed with two intermediate electron light areas. In the present work, only three-layered structures were seen in the outer membrane of the leptospires.

The mechanically disrupted antigen used in this work released damaged axial filaments and outer membranes, several of which were seen close to the protoplasmic cylinder. In addition, some of the outer membrane and blebs were still intact on the leptospires that still retained the original spiral form. The presence of some outer envelope on the leptospire indicates that the technique was probably not very efficient at removing this part of the leptospire. The outer membrane has been shown to have diagnostic and immunogenic properties (Auran *et al.*, 1972, Trueba *et al.*, 1990).

At present there is a paucity of information on the ultrastructure of the mechanically disrupted antigen although the use of this antigen in the ELISA has been reported by Trueba *et al.* (1990). Those workers found that more of this antigen was required to optimise the ELISA plate for serological work than the SDS solubilized antigen. This might be related to the parts of leptospire present in the antigenic preparation. In the present work, it was observed that the removal of the outer membrane was incomplete in the mechanically disrupted antigen.

The overall protein yield from the leptospire is proportional to the quantity of the lipopolysaccharide (LPS) in the outer envelope. These LPS have been shown to be the immunodominant antigen in leptospires, however the yield is usually poor (Yan *et al.*, 1999). Differences were observed in the quantity of protein assayed from the four test serovars with *L. hardjo* being the lowest. There are differences in the generation time of leptospires (Johnson and Faine, 1984) and length and wavelength of leptospires are strain dependent (Hovind – Hougen, 1986).

There is need for comparative study of the two types of antigens in immunoprotection and immunodiagnostic work.

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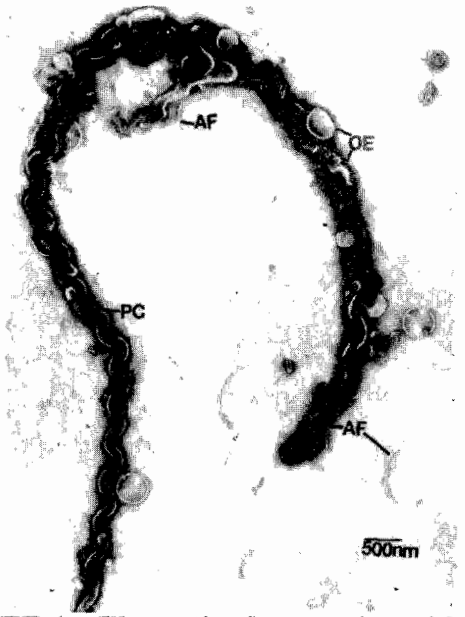

PLATE 1: The entire Leptospire with slight mechanical disruption (*L. pomona*)

AF = Axial filament
PC = Protoplasmic cylinder
OE = Outer envelope.

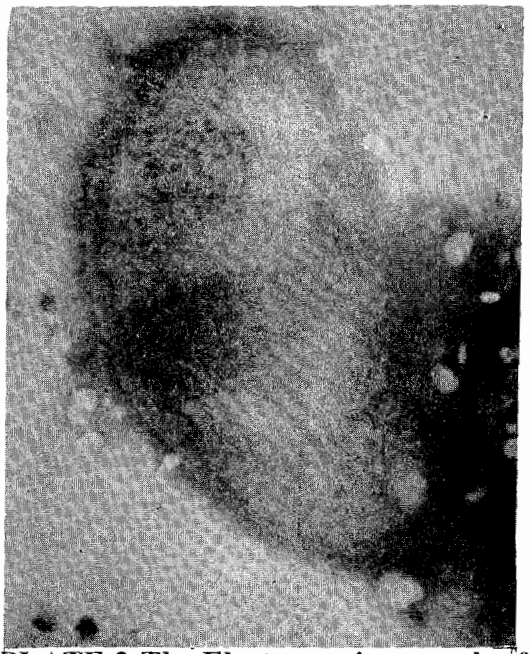

PLATE 2:The Electron micrograph of the salt altered *L. hamptoni*

White bar = 100nm.

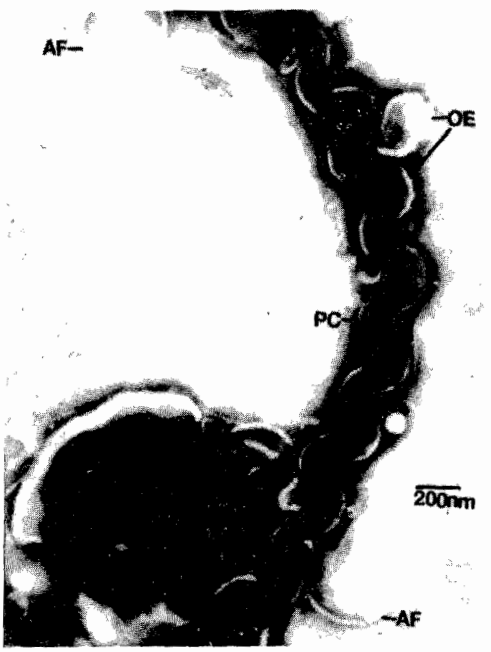

PLATE 3 : Mechanically disrupted *L. hamptoni*

AF = Axial filament
PC = Protoplasmic cylinder
OE = Outer envelope.

TABLE I: Protein yields of SDS-solubilised and mechanically disrupted (MD) leptospires

Culture No.	Protein yield (ug/ml) by leptospires							
	grippotyphosa		*hamptoni*		*hardjo*		*pomona*	
	SDS	MD	SDS	MD	SDS	MD	SDS	MD
1	322.71	248.27	259.82	208.12	186.62	179.29	380.56	290.60
2	325.09	254.37	276.12	226.65	195.22	152.21	347.94	258.32
3	308.22	225.42	252.67	216.37	199.47	167.17	389.83	247.37
4	296.31	249.38	235.92	209.19	205.24	149.28	310.42	296.29
*Mean	315.58[a]	244.36[a]	256.13[c]	215.05[c]	196.63[d]	161.99[d]	257.19[e]	273.15[e]
SD	16.43	12.90	16.67	8.53	7.84	13.94	35.98	23.98

SD = Standard deviation

* means with the same superscript within the same serovar are not significantly different (P>0.01)