Full Length Research Paper

Experimental *Yersinia pseudotuberculosis* enteritis in laboratory animals

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The course of *in vivo* pathogenicity of *Yersinia pseudotuberculosis* in groups of rabbits and gunnea pigs were examined. One group of the animals was infected orally with 10^8 cfu/ml of test organism and the second group with standard reference strains. The third group was dosed with clean water as negative control. Both controls and *Y. pseudotuberculosis* infected animals were closely monitored for clinical signs for three weeks during which loss of body weight, rise in temperature, ruffling of fur were noticed. Pure isolates of the organisms were re-isolated from the faecal samples of the infected rabbits and guinea pigs. Animals orally fed with clean water showed no symptoms of yersiniosis. Rabbits infected with *Y. pseudotuberculosis* showed signs of illness while guinea pigs did not show any clinical sign. Visceral organs of infected rabbits showed enteritis with necrotic lesions but no pathological changes were observed in all guinea pigs including the controls. In the clinically ill animals, tissues analyzed demonstrated polarized profile and inflammatory cell influx throughout the course of the test. These findings should assist the Veterinary Pathologist recognize suspected cases of enteritis due to *Y. psuedotuberculosis* in the field among similar animal species.

Key words: Rabbits, enteritis, *Yersinia pseudotuberculosis*, experimental infection, Nigeria.

INTRODUCTION

The term yersiniosis refers to infection caused by either *Yersinia enterocolitica* or *Yersinia pseudotuberculosis,* which appears as enteritis and sometimes septicemia in human and animal (Hurvell, 1981; Mair, 1973). *Y. pseudotuberculosis* occurs in water and in the environment and has been found in various wild and domesticated animals (Fukushima et al., 1988; Hayashidani et al., 2002; Inoue et al. 1988; Pocock et al., 2001; Toma, 1986). *Y. pseudotuberculosis* causes various clinical syndromes in human and animals. To induce disease, a set of virulence factors related to plasmid and chromosomal genes must be present which contribute to host colonization and prevent the action of specific and non specific host defense mechanisms (Martins and Facao, 2004).

Pathogenic *Y. pseudotuberculosis,* like other pathogenic *Yersinia* species, harbours a plasmid of 70 - 75 kb,

known as the *Yersinia* virulence plasmid pYV (Brubaker, 1991). The products of the genes on this plasmid are grouped into 4 main classes namely; adhesion/invasion protein (YadA), antiphagocytic secreted proteins (YOps), proteins involved in YOP processing and secretion (Ysc) and regulatory proteins (LCr) (Salyers and Whitt, 2002). Invasiveness is mediated by the gene inv on the chromosome and gene yadA on the plasmid (Revell and Miller, 2001).

The inv gene is thermoregulated and it encodes for a 103 Kdal protein, invasin, which binds to specific receptors on mammalian cells and facilitates the entry of *Y. psuedotuberculosis* into tissues (Iserberg and Leong, 1988). Another very important characteristic of *Yersinia* virulence mediated by chromosomal genes is the ability to capture iron which is regulated by a set of genes forming the high- pathogenicity island (HPI) (Carniel, 1999). Interaction between bacteria and host cells is an important step in the establishment of infection. Hence after intestinal colonization by pathogenic *Yersinia*, the

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bacteria penetrate the intestinal mucosa through the m-cells. *Y. psuedotuber-culosis* colonize peyer's patches disseminating through the lymphatic pathway and eventually reaching the mesenteric lymph nodes, the liver and the spleen (Grutz-Kau et al., 1990; Falkow et al., 1992; Clark et al., 1998).

The pathogenicity of *Yersinia* can be studied by *in vivo* experimental infection and monitoring its kinetics in animals such as mice, rats, rabbits or guninea pigs. The latter has been well documented in *Y. enterocolitica* (Quan et al., 1974; Falcao et al., 1984; Apfel and Noleto, 1991; Hessemann et al, 1993; Okwori et al, 2005). However, only a limited *in vivo* infection of *Y. pseudo-tuberculosis* and the kinetics has been studied (Martin et al., 2004). This study is aimed at determining the pathogenic potentials and indicators of *Y. psuedotu-berculosis* in rabbits and guinea pigs.

MATERIALS AND METHODS

Bacterial strain

Y. pseudotuberculosis strains were obtained from human and animal sources in Nigeria (Okwori et al., 2005).

Source of rabbits and guinea pigs

Bacteriologically controlled, six New Zealand breed of rabbits weighing between 500 – 750 g were obtained from the small animal Laboratory of the National Veterinary research Institute, Vom, Nigeria. Abyssinian breed of guinea pigs weighing 200 – 400 g were also obtained from the same source.

Experimental design

The animals were housed in separate cages (45 by 46 by 32 cm). They were first observed and acclimatized to laboratory conditions for a period of 9 days. Prior to the experiment the animals were monitored for 4 weeks for any noticeable clinical symptoms. Faecal samples were screened bacteriologically to ensure the absence of the *Y. pseudotuberculosis*. The animals were serologically screen-ed for *Y. pseudotuberculosis* antibodies prior to experiment to ensure that they were free of antibodies to *Y. pseudotuberculosis.* Each cage was identified by a letter and each animal (rabbits and g/pigs) was identified by a number. The body temperature was recorded daily to ensure that the animals remained afebrile. Drinking water was available *ad libitum*, and diet distributed every morning. The animals were divided into 3 groups with each containing 2 rabbits and 2 guinea pigs. The groups were labelled A, B, C. Each group of the animals were housed separately in a roofed block building with cross ventilation, in an environmental tempera-ture of 25 ± 1℃ and relative humidity of 50%. Group A were infected with Nigerian isolates of *Y. pseudotuberculosis*, group B with reference strain of *Y. pseudotuberculosis* (positive control) and group C with sterile distilled water (negative control). The route of inoculation was oral. Grower mash was used for feeding the animals within the period of the study. The reference control *Y. pseudotuberculosis* strains were obtained from the Faculty of Veterinary Medicine, University of Helsinki, Finland. Animal care and manipulations were conducted in accordance with the Consortium Guide (1988).

Preparation of inoculum

Aliquots (50 µl) of a fresh culture of *Y. pseudotuberculosis* (local and control strains) grown separately in 2 bottles of tryptic soy broth (TSB) at room temperature were plated on tryptic soy agar (TSA). After 20 h of incubation at 20°C, bacteria colonies were scraped from the plates and washed in phosphate buffered saline. The tur-bidity of the suspension was adjusted to an optical density of 1.0 at 600 nm, corresponding to 1.6×10^9 cfu/ml (Delor and Cornelis, 1992). The bacterial viable count was checked by plate enume-ration (Miles and Misra, 1938).

Per oral challenge of animals

Animals in groups A - B were deprived of drinking water for 24 h and then challenged by allowing them to drink from aqueous bacterial suspension containing about 1.6×10^9 cfu/ml for 24 h. The inocula were then withdrawn and the animals served with clean water after 24 h. Those in group C were served with sterile distilled water. The animals were observed for weight loss, signs of diarrhoea and death for 3 weeks. During this period, faecal samples were collected from both species of animals (rabbits and guinea pigs) at weekly intervals for the culture. The animals were sacrificed (by inhalation of chloroform), dissected and the viscera organs examined after three weeks. The heart blood was also cultured to reclaim the organisms in pure cultures. The sera from all infected animals were reacted against both local and reference control organisms. Each rabbit and guinea pigs were weighed once every 2 or 3 days and evidence of diarrhoea assessed daily.

RESULTS AND DISCUSSION

Six days post infection, rabbits in groups A and B showed signs of depression and isolation. This was immediately accompanied with progressive ruffling of the fur as well as steady loss of weight (Figure 1). Body temperatures were also seen to increase (Figure 2). The organisms were recovered in large quantities from the stool culture on selective culture medium.

Post mortem result showed severe necrotic lesions which repleted the whole matrix of the liver tissue with some hepatic hypertrophy. Other observations included enlarged lymph nodes especially the mesenteric lymph nodes, pigmented lungs and renal hypertrophy. Signs of enteritis were seen on the visceral organs of the infected rabbits.

Y. pseudotuberculosis infection in man animal is characterized by emaciation, ruffling of the fur, septice-mia, and severe abscesses, on the viscera as well as mesenteric adenitis (Topley and Wilson, 1990). The results presen-ted in this study show the ability of the micro-organisms to invade and multiply in the infected animals. This is directly related to the presence of the pYV plasmid regardless of the inoculation route as previously documented by Martins and Facao (2004). The ability of *Y. pseudotuberculosis* to neutralize the effects of the host's immune system and thus survive and spread through the spleen and liver depends on whether it harbors this plasmid.
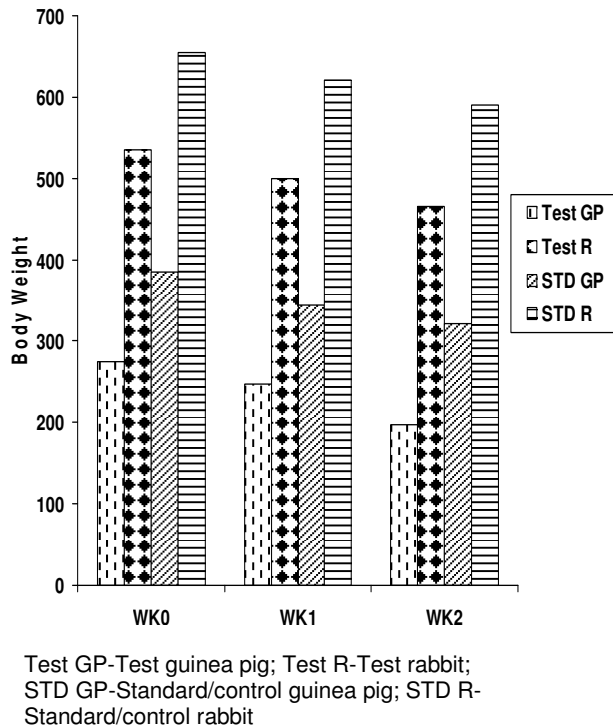
Our result is similar to the findings of Topley and Wil-

Test GP-Test guinea pig; Test R-Test rabbit;
STD GP-Standard/control guinea pig; STD R-
Standard/control rabbit

Figure 1. Variations in body weight of animals inoculated with
Yersinia pseudotuberculosis.

Test GP-Test guinea pig; Test R-Test rabbit;
STD GP-Standard/control guinea pig; STD R-
Standard/control rabbit

Figure 2. Temperature rise in animals inoculated with *Yersinia
pseudotuberculosis.*

son (1990) that *Y. pseudotuberculosis* infection is characterized by emaciation, ruffling of the fur, septicemia and severe abscesses on the viscera as well as mesenteric adenitis.

The absence of diarrhoea in animals infected with *Y. pseudotuberculosis* may be due to lack of heat stable enterotoxin as in *Y. enterocolitica* (Boyce et al., 1979). The necrotic lesions observed in the viscera of animals infected with *Y. pseudotuberculosis* as well as their isolation in pure cultures from these organs agree with the findings of Krauss and Hensel (1961) and Wise and Uppal (1972). Hence our findings are suggestive of the invasive nature of the pseudotuberculosis toxin responsible for tumour formation (Gemski et al., 1980; Portnoy et al., 1984). It also reveals the potency of a chromosomally encoded protein called invasin (inv) in the outer membrane that facilitated the intracellular penetration of the above mentioned toxins (Isberg and Falkow, 1985). It may be interesting to note that *Y. pseudotu-berculosis* has the viscera as their predilection site, unlike *Y. enterocolitica*. *Y. pseudotuberculosis* has been seen to produce lesions on the viscera of infected rabbits under investigation hence could be said to be highly invasive. This is different from the reports of Schleifstein and Coleman (1939).

Animals used as negative controls showed no symptom and hence gained weight as documented in a similar study by Delor and Cornelis (1992). These findings should help veterinarians recognize suspected cases of
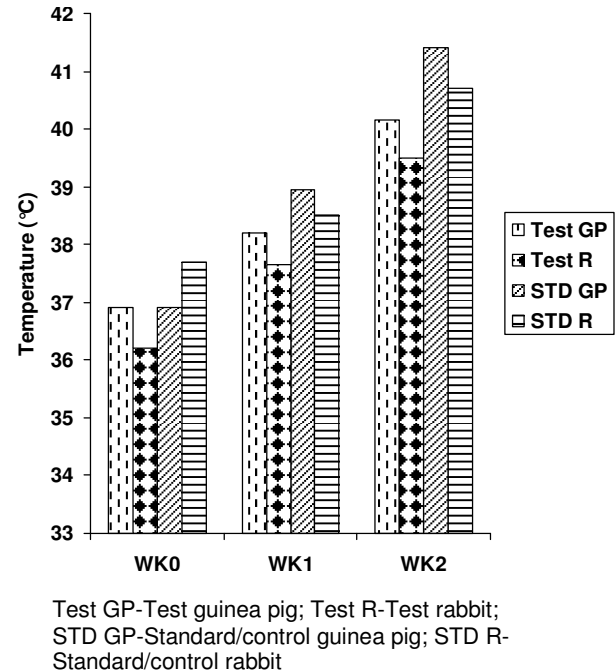
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ACKNOWLEDGEMENTS

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