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Weight and Temperature Trends of Sprague Dawley Albino Rats Injected *B. bacteriovorus* (ATCC® 15364™) and Pathogenic Bacteria

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ABSTRACT

To investigate effects on the physiology of mammals, 144 Sprague Dawley rats, were used as models to monitor changes in average weights and temperatures over a 168 hour period. These were sub- divided into 11 groups, each composed of 12 rats each. Five groups (n=12 per group) were injected with each of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. multocida* and *P. vulgaris.* Another 5 groups (n=12 rats per group) were each injected with the bacterial pathogens outlined, but also injected with a suspension of one ml of 1 x 10^8 *B. bacteriovorus* (ATCC® 15364™). A group, (n=12), rats, un-injected with neither pathogen(s) nor *B. bacteriovorus*, served as control. Temperatures and weights were taken on a daily basis and the averages obtained. Results showed that all rats groups, before pathogen injection had higher average weights compared with lower weights observed post injection with pathogens. Similarly, average temperatures in rats were higher pre-pathogens injection, compared with lower values post-injection. On comparison of groups injected with pathogens with groups injected with both pathogens and *B. bacteriovorus*, there was no significant difference in temperatures, though the latter group showed lower average temperatures. *B. bacteriovorus* (ATCC® 15364™) injected into rats showed no significant changes in weights nor temperatures between them and the control group. It is concluded *B. bacteriovorus* has no apparent effect on temperature and weight of rats when injected into them.

Keyword: *B. bacteriovorus* (ATCC® 15364™) , weight, temperature, rats, pathogens, changes

INTRODUCTION

Bdellovibrio, like viruses, are sub-microscopic, because they are smaller than other bacterial species. *Bdellovibrio* are Gram-negative, vibroid bacteria that range between 0.3 - 0.5μm by 1.4 - 2.5μm in size (Harini, *et al*., 2013).

Bdellovibrio are highly motile, single, polar, sheathed flagella which are sheathed. They are predatory and feed on other Gram-negative bacteria, including animal and plant pathogens. During attack, they use degradative enzymes, to create pores in the host cell walls to access the periplasm, where they use prey cytoplasmic contents as nutrients for growth and reproduction. Finally they burst open the host cell envelopes, causing their deaths (Kilpatrick, 1999; Kadouri and O'Toole, 2005).

This bacterium is of interest to microbiologists because it can be potentially harnessed as living controls of many pathogenic Gram-negative microorganisms. Secondly, studies on their degradative enzymes give insight into targets in prey cells that are evolutionarily successful points of attack and could be used for design of antimicrobial agents (Lambert *et al.*, 2006).

Though other predatory bacteria such as *Micavibrio*, have been evaluated for predatory activity, *Bdellovibrio* shows advantage over other them. While *Micavibrio,* for example, leeches to its host as it feeds, *Bdellovibrio* which feeds on its prey from within, are able to adapt to changing prey resistance strategies and conditions (Wang *et al*., 2011).

Species of *Bdellovibrio* are present in the tropical Nigerian aquatic and terrestrial environment (Sar *et al*., 2015; Sar *et al.*, 2016). It is hoped that they could be harvested and harnessed in the fight against pathogens of man and animals.

MATERIALS AND METHODS

One hundred and forty-four (144) Sprague Dawley (SD) albino rats, approximately 16 weeks old were used. They were housed under ambient temperatures of about 25^{o}C ± 5^{o}C, and Relative Humidity (RH) of about 55% ± 5%. Feed and water were supplied *ad libitum.*

To assess the effects of *Bdellovibrio* on rats, one group (n=12) rats was injected with 1 ml 10^8/ml *B. bacteriovorus* ((ATCC® 15364™). The test bacterium, *B. bacteriovorus* (ATCC® 15364™) was prepared and maintained according to the enclosed instructions of the ATCC® and the product sheet (ATCC®, 2018).

To evaluate effects of respective pathogen on rats, five groups (n=12 rats per group) were subcutaneously injected with 1 ml 10^8/ml of *E. coli*, *P. multocida*, *P. aeruginosa*, *P. vulgaris* and *K. Pneumoniae* for 168 hours.

For documenting effects of *B. bacteriovorus* on *in vivo* pathogens, another group of rats (n=12 rats per group) was again subcutaneously injected with a pathogen, then injected with 1 ml (1 x 10^8 CFU/ml) *B. bacteriovorus* (ATCC® 1534™) suspension for 168 hours.

All experimental rat groups had their weights and temperatures measured once daily, at approximately the same time periods, for the duration of the study.

DETERMINATION OF BACTERIAL PATHOGENICITY

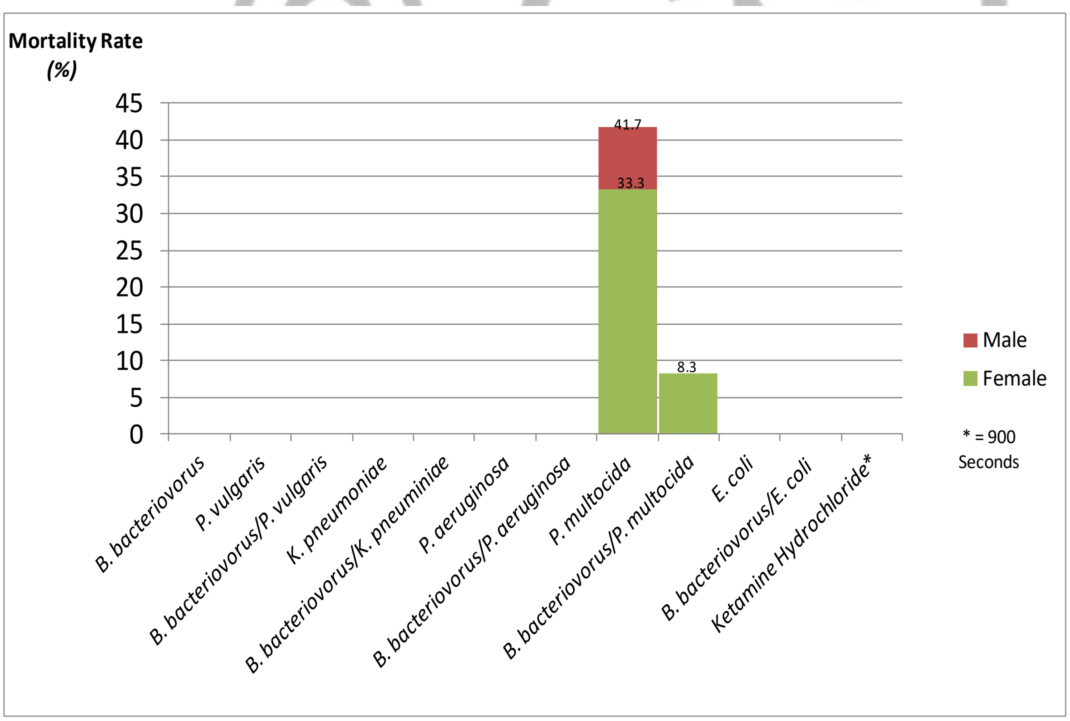
Test bacteria were serially diluted in physiological saline. The CFU of bacteria in each dilution was determined using McFarland's standard. Six animals each were subcutaneously injected with the dilutions of inocula. Mortality was considered a sign of infection. Otherwise morbidity was determined by blood cultures and ubPCR. The ID_{50} was calculated according to the method of Reed-Muench as reported by Saganuwan (2015).

STATISTICAL ANALYSIS

Data were analysed using IBM SPSS version 23.0 (2015). ANOVA was used to test to test for group differences in weights and temperatures. All tests were considered significant at $p \leq 0.05$ level of probability.

RESULTS

Though no change in the temperature was observed, a mortality rate of 75.0% (n=9; 5 male and 4 female) was recorded in rats injected with *P. multocida* and 8.3% (n=1 female rat) in those injected with *P. multocida* and then with *B. bacteriovorus* **(Figure 1)**. Weight trends showed that the rats increased by 5.8 g after *P. multocida* and *B. bacteriovorus* injection.



Source: Sar *et al.* (2018)

Fig. 1: Mortality Rates in Sprague Dawley Rats Injected with Gram-Negative Pathogenic Bacteria and with *B. bacteriovorus* and Pathogen

Weight Changes

Figure 2 summarises weight change findings. Within the five groups of rats injected with pathogens, four groups, 48 rats (80%) showed reduction in average weight, while one group of 12 rats (20%), increased in average weight, post intra-peritoneal pathogen injection. In the group of rats injected with both *B. bacteriovorus* and pathogens, three groups; 36 rats (60%) showed loss in average weight, while two groups; 24 rats (40%) increased in average weight.

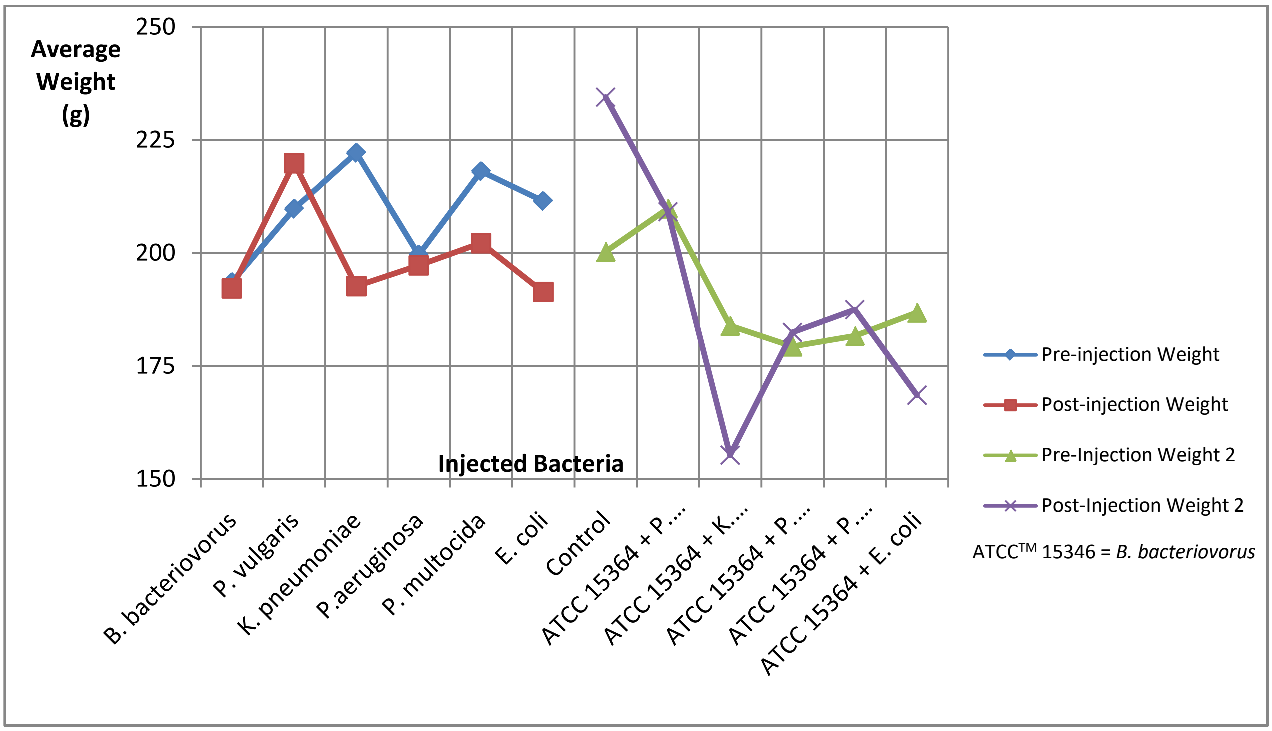


Fig. 2: Weight Changes in Sprague Dawley Rats Injected with Pathogens and Pathogens + *B. bacteriovorus* (ATCC® 15364™)

However, no significant differences were found between rats injected concurrently with *B. bacteriovorus* and pathogens and those injected with only pathogens.

Curiously, the group injected with only *B. bacteriovorus* showed a decrease of 1. 70 g in weight. However, as expected, the untreated control group increased in weight by 32. 2g.

Figure 3 details changes in SD rats injected with bacterial pathogens, and with pathogens and *B. bacteriovorus* (ATCC® 15364™). It shows that out of the five groups of rats injected with only pathogens, one group, 12 (20%), injected with *P. aeruginosa*, showed increase in average temperature. The other three groups, 36 rats (60%) showed decrease in average temperatures, by as much as 2.7°C in the *E. coli* injected rats. In the *P. multocida* injected group, the temperature remained unchanged.

In the rats injected with both pathogen(s) and *B. bacteriovorus*, the highest temperature change of 0.8 °C, observed pre- and post- bacterial injection was in the *B. bacteriovorus* and *K. pneumoniae* injected group.

In the *B. bacteriovorus* injected rats, a slight increase of 0.8 °C was recorded, while in the untreated control group, a marginal decrease of 0.1 °C in average temperature was observed.

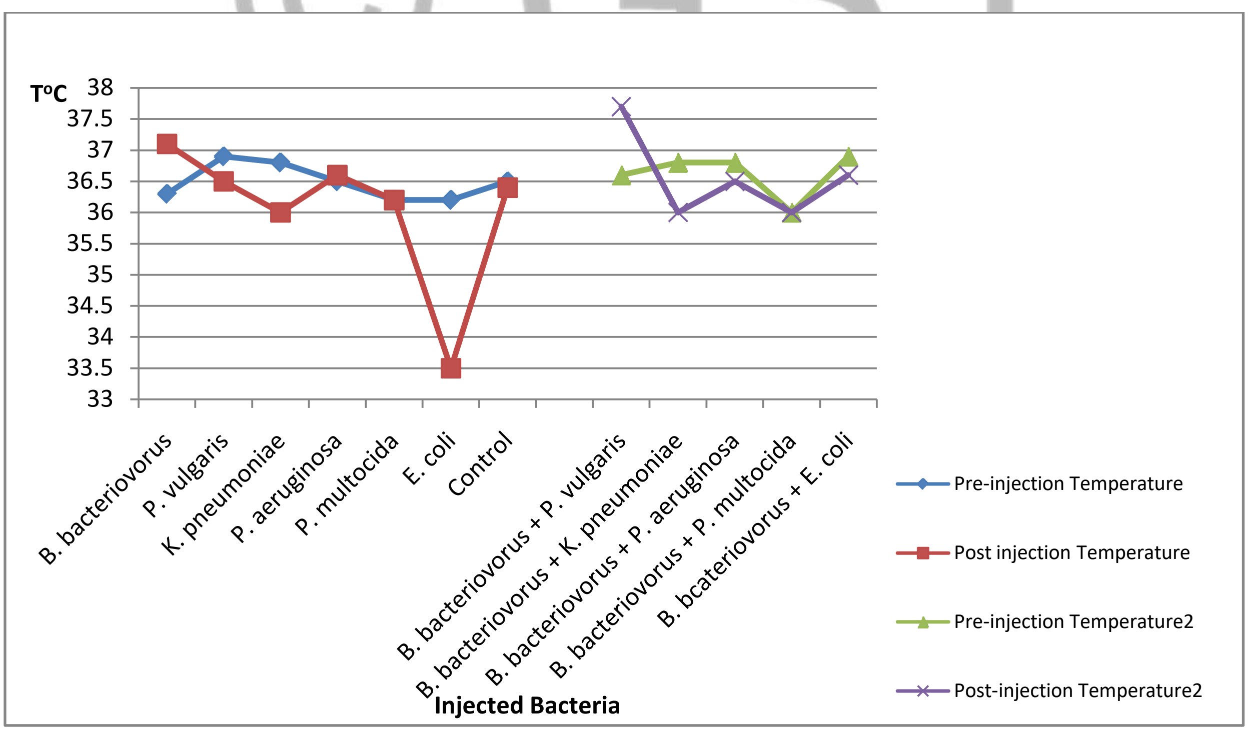


Fig. 3: Temperature Changes in Rats Injected with Bacterial Pathogens and *B. bacteriovorus*

DISCUSSION

Both weight and temperature are indicators of the proper homeostatic functioning of an organism, especially mammals. Within optimal limits, both show that a mammal's n animal's internal physiology is working functioning normally (Martinez del Rio and Karasov, 2010).

This investigation into the effect of *B. bacteriovorus* (ATCC® 15364™) injections on the weight of SD rats indicated that the bacterium may not have had any significant impact on their weights, as evidenced by the marginal variations in weight of rats injected with only *Bdellovibrio*.

Similarly, the weights of pathogen-injected rats were generally lower when compared with weights of rats injected with both pathogens and *B. bacteriovorus*. This could mean that *Bdellovibrio* may have moderated the morbid effects of injected pathogens on the rats, as reported by Monappa *et al.* (2016). Similarly, Shilpi *et al*. (2016) also noted that as part of the commensal flora of the gastro-intestinal tract of animals, *Bdellovibrio* helps to keep the population of other bacteria, including pathogens and potential pathogens in check. In this role, they have no observable negative effects on the host animals. Therefore, it is unlikely that in this study they could have had any adverse impact on rat weight metabolism.

However, that there were observed losses in weights (at all) in rats injected with both pathogen and *Bdellovibrio* could be attributed to pathologic and physiologic changes caused not by *Bdellovibrio*, but by pathogens. Such physiologic changes could have led to lower feed consumption, or even altered absorption of ingested feed and water in the gastro-intestinal tracts of these rats. Since feed and water were provided *ad libitum*, the only conditions that varied and may have caused changes in weights were the injected pathogens, and pathogen-*Bdellovibrio* injections. As previously postulated, if injections of only *Bdellovibrio* apparently caused no significant variations in weights, a logical conclusion would be that any change (in weight) could be attributable to pathogen activity.

Regarding temperature, it was expected that injection with pathogens would cause rat temperatures to rise, as these would act as pyrogens, according to one pioneering study by Gudjonsson (1932). However, there was no observed clear pattern of temperature related responses in pathogen-injected rats. Kluger *et al*. (1998) advised that interpreting hyperthermia events in rats should be approached cautiously, as their fever response is complex. This response complexity was demonstrated in rats injected with *P. multocida.* The seventy-five percent mortality in these rats suggested that the *P. multocida* being highly virulent to the rodents, caused extreme morbidity. Such morbidity should have led to observed increases in temperatures. However, even in these rats, no changes in temperature was observed before treatment, and at the end of measurements. Even though no explanation for this phenomenon is handy, the significant statistical difference in temperature between *P. multocida* injected SD rats and those injected with *Bdellovibrio* and *P. multocida* suggest that *B. bacteriovorus* lessened the effects of this otherwise virulent pathogen to the extent that the temperatures dropped leading to the recorded differences. Further, Tocco-Bradley *et al*. (1985) stated that an animal under stress may not show a febrile response for various reasons, including the release of gluco-corticoids or prostaglandins, (substances called endogenous pyrogens), which suppress fever development. In this study, the lower temperatures observed on injecting rats with both *Bdellovibrio* and pathogens could be that the higher bacterial load of the two organisms placed greater stress on the rats and caused production of endogenous pyrogens which led to lower temperatures.

CONCLUSION

This study undertaken to determine and to assess the effects of *B. bacteriovorus* on animals when used as control for some common systemic Gram-negative bacterial infections has demonstrated that *Bdellovibrio bacteriovorus* preparation injected into rats inoculated with pathogenic Gram-negative bacteria moderated the adverse physiologic effects of the pathogens on the rats as monitored by weight and temperature responses. However, there is need for further investigation, such as assessing the histo-pathologic responses to the organism, so that its full *in vivo* anti-pathogenic bacteria potentials can be safely harnessed.

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