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Faecal Shedding of *Arcobacter* species following experimental infection in rats: Public Health Implications

Research Article

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Abstract: *Arcobacter spp.* are emerging food borne pathogens associated with prolonged diarrhea and occasional systemic infections such as bactereamia and peritonitis in humans. Information on faecal shedding patterns to assess the potential role they play within the intestine however, is lacking. This study was designed to investigate faecal shedding of local isolates of *Arcobacter spp.* Using real time PCR for confirmation, *A. cryaerophilus* and *A. butzleri* were isolated from the stool of healthy chickens. Pathogenicity of the organisms was tested by administering a single oral challenge of 102-109 cfu/ml to 45 healthy adult male albino rats divided equally among 5 groups. Uninfected rats were used as the control group. *A. cryaerophilus* and *A. butzleri* produced infection in 100% of the animals. Experimental infection was dose dependent and caused diarrheal illness and faecal shedding was noted up to 5 weeks post infection. The present study demonstrates that rats can act as a reservoir and potential source of *Arcobacter* infection in humans and animals exposed to this pathogen.

Keywords: *Arcobacter* • *Feacal shedding* • *Public health*

1. Introduction

Arcobacter spp. are emerging as enteropathogens with increasing evidence for zoonotic potential having been described world wide in food of animal origin [1], environmental samples [2], and in humans and animals [3]. Despite increased awareness and isolation in cases of gasteroenteritis, the source of these infections in humans is still unclear [4]. Consumption of contaminated food or drinking water has been suggested as the possible sources of infection [5]. *Arcobacter* species have been isolated from various animals with diseases including abortion, septiceamia mastitis, gastritis and enteritis [6] as well as in the faeces of healthy livestock [7]. *Arcobacter spp.* may be present in the digestive tract of healthy livestock in the absence of clinical signs of illness [8,9].

Evidence of intestinal colonization by *Arcobacter* organisms, specifically isolation of *A. cryerophilus* from healthy humans, has been reported [10].

Besides the reports of *Arcobacter* spp. in humans and animals, the presence of the organisms in both ill and healthy non human primates has also been described [11].

Natural *Arcobacter* spp. distribution in rodents is unknown however, experimental infection in mice, guinea pigs, hamsters and rabbits produced neither clinical symptoms nor pathological lesions at necropsy. [12]. The fact that *Arcobacter* spp. have been frequently isolated from animals, food of animal origin, drinking water, plants, and the environment is strong evidence in support of its zoonotic potential [13]. Livestock in particular may act as a significant reservoir of *Arcobacter spp.*

Faecal shedding of a bacterial strain has been described as an indication that its presence in the alimentary tract is detected by culture of faeces or cloacae swabs. The aero tolerance properties of *Arcobacter* enables the bacteria to survive and spread in the environment for a long period [14].

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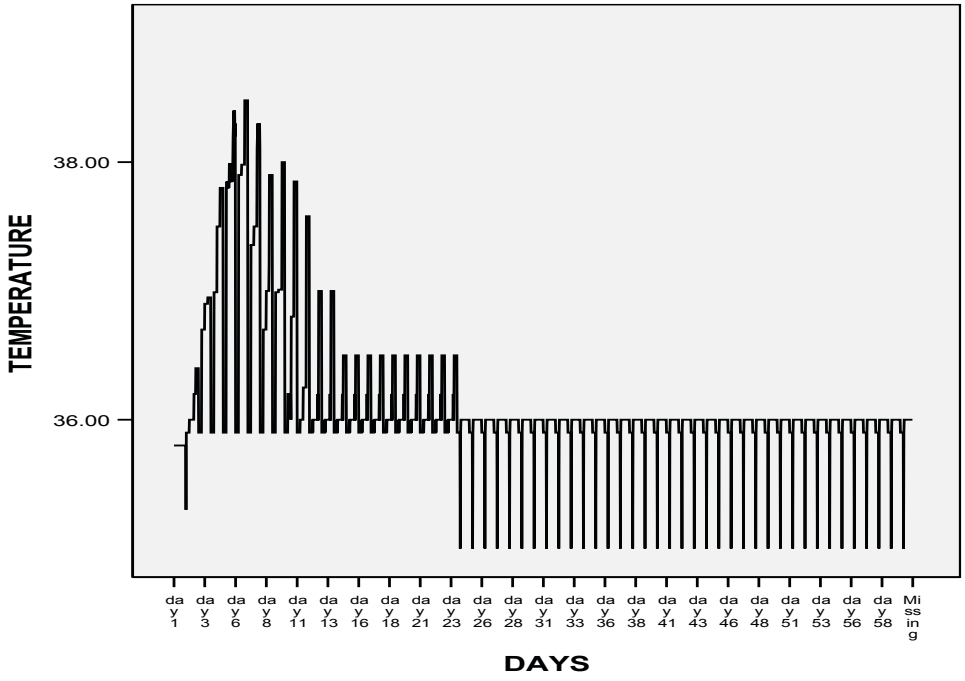
Figure 1. The curves for normal rats (36°C on day 1) and temperature of infected rats from days 2-25 there was bactereamia and eventually became normal from day 25 through day 60.



The pattern of faecal shedding in an animal model may play a part in the epidemiology of zoonotic bacterial infections. From the public health perspective, the faecal carriage of food borne pathogens among livestock that share the same environment with humans is strongly correlated with the risk of *Arcobacter* transmission to the human population. Such may be the case in developing countries such as Nigeria. This study therefore aims to determine the relative pathogenicity of *Arcobacter* in an experimental rat model based on the duration of faecal shedding and intestinal colonization post inoculation with a view of providing insight into the potential risk of environmental contamination.

2. Material and Methods

2.1. Animals

Forty-five male, five-month-old healthy albino rats (*rattus novegicus*) weighing 200-250g were acquired from the animal house unit of the College of Health Sciences, LAUTECH, Osogbo, Nigeria. The rats were housed in 33 x 20.5 x 19 cm transparent plastic cages and were acclimated to the new environment and human handling. The animals were fed an antibiotics free ration and given water ad libitum. They were divided into groups of 5 rats per cage and each group received a similar dosage of the *Acrobacter* inoculum ranging from 10^2 -10^9 CFU per ml. The control group were tagged before inoculation and received sterile saline. Assessment of the state of health of the animals was based on feed intake, consistency of faeces, rectal temperature, and the presence of rough skin. Feacal cultures were performed to rule out previous infection with *Arcobacter* organisms. All the animals were handled and treated according to international experimental animal ethics rules.

2.2. Preparation of *Arcobacter* inoculums

Strains of *Arcobacter butzleri and A. cryerophilus* - originally confirmed by real time PCR and maintained in stock cultures at -25°C glycerol *Arcobacter* broths - were resuscitated in brain heart infusion agar supplemented with 5% yeasts and 7% sheep blood and incubated at 35°C in microaerophilic atmosphere [5]. For preparation of the suspensions, bacteria was collected at exponential growth stage and diluted in 0.95% normal saline. The broth was then standardized by Mc Farlands Nephelometry ranging from 10^2-10^{10} CFU per ml.

2.3. Animal inoculation

For animal inoculation, 1ml of *Arcobacter cryaerophilus* and *A. butzleri* suspension containing 10^2-10^{10} CFU (colony forming units) was given orally to the rats with the 1ml sterile syringe. 1ml of sterile normal saline was given to the other sets of 5 rats who acted as the control group.

Figure 2. Over a period of 6 weeks, *Arcobacter* cryerophilus was shed in the faeces of experimentally infected rats at a 900% colony count while *A. butzleri* was shed at a 400% colony count.

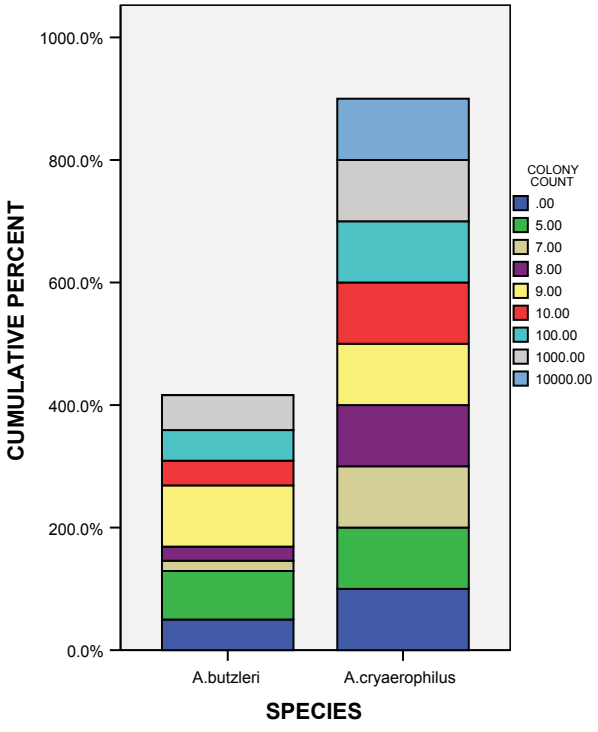
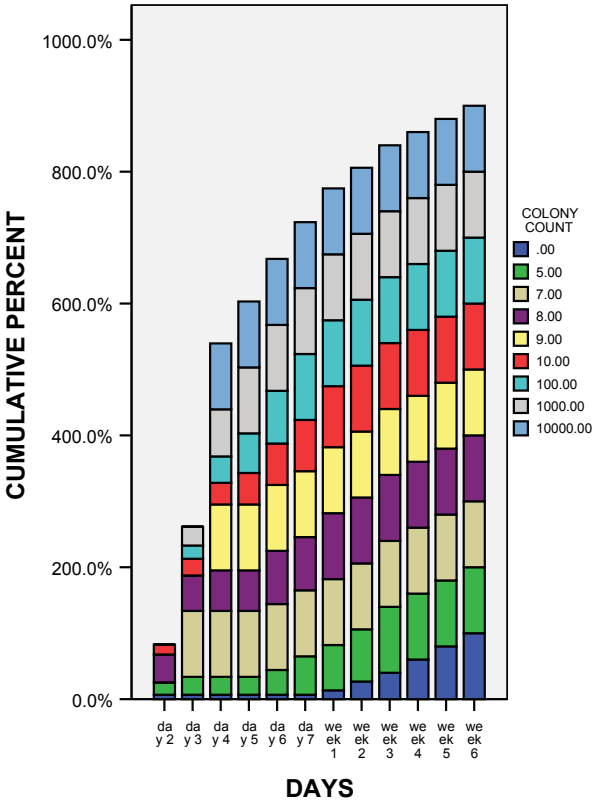


Figure 3. Feacal shedding of *Arcobacter* was at peak within 3 weeks of infection and reduced to 0% progressively after 4 weeks until 6 weeks post infection.



3. Results

Reduced activity, loss of weight, reduced appetite, rough coat and obvious diarrhea was observed in all the rats in the infected group. The diarrheic stool was loose and contained mucus but no blood was seen with or without a microscope. Diarrhea appeared to be self limiting after 3 weeks without therapeutic intervention. Bacteramia of infected rats from days 2-25 progressively increased and eventually became normal from day 25 to day 60 (Figure 1) A dose dependent response was observed with higher doses producing a corresponding increase in bactereamic temperature (Figure 2) The control groups were healthy throughout the study period.

All of the animals in the infected group shed *Arcobacter* in their stool for at least five weeks even when clinical diarrhea was no longer evident. A mean viable recovery count of $1.6x10^2$ to $4.7x10^7$ cfu/g of faeces (SD: $4.2x10^7$cfu/g) was observed in the rats infected with the *A. butzleri* inoculum starting at 2 days post-infection until the fifth week of sampling. The rats infected with the *A. cryaerophilus* inoculum had a mean viable recovery count of 2.4x10 to $3.65x10^7$ cfu/g of faeces (SD: $1.3x10^2$ cfu/g). *Arcobacter cryerophilus* was shed in the faeces of experimentally infected rats at a 900% colony count while *A. butzleri* was shed at a 400% colony count over a period of 6 weeks (Figure 2).There was a significant difference in the mean number of *A. butzleri and A. cryaerophilus* in cfu/g of faeces cultured from animals given the same dose of bacteria ($P< 0.05$) as shown in Figure 2. Peak feacal shedding of *Arcobacter* was observed within 3 weeks of infection and reduced to 0% progressively after 4 weeks until 6 weeks post infection. Faecal samples from the control group were negative throughout the period of the experiment.

4. Discussion

In the environment, *Arcobacter* remains viable at 4°C for up to 3 weeks in the faeces of cattle, 4 weeks in water and 5 weeks in urine [15]. Existence of *Arcobacter* in the intestines of healthy livestock may contaminate the environment and the human food chain, posing a potential public health hazard [16].

Experimental infection of *Arcobacter* in albino rats had been previously established during the pilot study earlier in this experiment (data not shown) in order to obtain the infective dose (ID50) for 10^3 cfu/ml

Arcobacter organisms. We found that 10^3cfu/ml of bacteria produced mild diarrheaic disease while 10^6-10^9 produced significant intestinal pathological changes in rats. This was the rationale for the varying doses of 10^2-10^9 cfu/ml used in this experiment.

In this study, oral challenge with 10^2 and 10^9 cfu/ml of *Arcobacter* resulted in increasing doses in the digestive tract of the rats until the fifth week post-infection. Clearance of the organism was observed as the weeks progressed (> 4 weeks). This phenomenon may be due to the experimental nature of the infection and the ability to fight infection in an otherwise healthy animal. Shedding of *Arcobacter cryerophilus* was more significant compared to *Arcobacter butzleri* (Figure 3) consistent with findings in a previous study in which *Arcobacter* was isolated from seven of 500 (1.4%) stool samples of healthy people with *Arcobacter cryaerophilus* as the only species present [10].

The ability of locally isolated strains of *Arcobacter* to colonize the intestinal tracts and produce clinical diarrhea observed in this study supports the pathogenicity of these isolates. Bactereamia response was demonstrated by the temperature chart suggesting the capacity of *Arcobacterspp.* to produce disease in rats, in contrast to a similar study of *Campylobacter* where colonized rats did not succumb to clinical diarrhea or pyrexia [17]. That study did highlight however a considerable risk for environmental contamination from infected household rats. Although the rats used in this experiment are relatively pathogen free, further studies in the household rats are essential in order to assess their role as potential reservoirs for *Arcobacter* spp.

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