Histopathological and functional changes in the testicles of albino rats experimentally infected with *Arcobacter butzleri*

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**Background.** *Arcobacter* is an emerging zoonotic enteropathogen associated with spontaneous abortion, diarrhea and mastitis in domestic animals.

**Materials and methods.** Thirty male pathogen-free albino rats were infected with a single challenge of *Arcobacter butzleri* (10⁸ cfu/ml) isolated from the stool of healthy pigs with the aim of investigating the effects of *A. butzleri* on rat testicular histology and spermatogenesis.

**Results.** In previously healthy male albino rats, *A. butzleri* caused testicular degeneration associated with reduced sperm count and motility.

**Conclusions.** The result of this study suggests that *A. butzleri* produces testicular degeneration and the associated disruption of spermatogenesis in albino rats; hence, its infertility potential in livestock industry and its economic importance should be further investigated.

**Key words:** *Arcobacter butzleri*, testicular degeneration, infertility, pathogenicity, sperm count, albino rats

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**INTRODUCTION**

*Arcobacter* is an emerging enteropathogen with an increasing evidence of the zoonotic potential associated with prolonged diarrhoea and occasional systemic infections such as bacteraemia and peritonitis in humans (1). An evidence-based semi-quantitative method for prioritization of foodborne zoonoses ranked *A. butzleri* as a microbe of “significant importance” (2). *Arcobacter* was first isolated from aborted bovine and porcine fetuses (3), especially *A. butzleri* and *A. skirrowii* which were isolated from aborted fetuses and placenta of bovine, porcine and ovine origin (4). Although no remarkable pathological changes were identified, the clinical signs associated with *Arcobacter* infection include infertility, chronic discharge during oestrus, stillbirth and late-term abortions (5).

The pathogenicity and virulence mechanisms of *Arcobacter* species are still poorly understood, despite its increasing incidence and isolation from clinical and food products (6). The virulence of *A. cryaerophilus* was also first described when it was observed that the strains tested induced the accumulation of fluid and electrolytes in the rat ileal loop assay and showed an *in vitro* invasion of Hep-2
cells (7). Several studies were reported on their adhesion capacity (8–10), invasiveness (9,11), and cytotoxicity (12–14) in several cell lines. Collectively, in these studies, the strains tested showed adhesion, invasion, and cytotoxicity, toxicity and adherence being the most commonly observed effects. The differences observed among different studies may be due to the origin of the strains (environmental versus clinical) as well as to different cell lines used in the studies. 

_A. butzleri_ being the most invasive species in experimental animal infections (15). Other animal models and _in vivo_ experiments have been reviewed in another recent publication (16). The presence of adhesion molecules in _A. butzleri_ have been proven by the capacity of the strains tested to agglutinate human, rabbit, and sheep erythrocytes, and hemagglutinin of about 20 kDa has been characterized by Western immunoblotting (17, 18).

A few animal studies on the pathogenicity of _Arcobacter_ species include experimental oral infection of caesarean-derived 1-day-old piglets in whom all strains colonized and multiplied in the gut of the tested groups, but only _A. butzleri_ strains (from human faeces and swine) were able to invade the internal organs of infected animals (19).

In another study, 3- to 5-day-old chickens and turkeys were infected orally with the human _A. butzleri_ strain. The results demonstrated the invasive and virulence capacity of the _A. butzleri_ strains, which was suggested to be host-dependent with respect to species and breed (20). Experimental infection in udder also produced clinical mastitis with fever and a reduced milk yield (21). In another study, venereal transmission was suggested as _A. butzleri_ and _A. cryaerophilus_ were isolated from the preputial fluid of boars and fattening pigs (22). _Arcobacter_ species were also detected in clinically healthy sows and live newborn piglets (23). Furthermore, different _Arcobacter_ species were detected in a single aborted fetus and in different fetuses from the same litter; these findings have indicated that the _Arcobacter_ strain plays the primary role in abortion and reproductive disorders while others are opportunistic pathogens (4). The role of _Arcobacter_ in chronic reproductive disorders in farm animals has not been previously investigated. Therefore, this study aimed to study the effects of _Arcobacter_ on the testes of albino rats with a view to provide an insight into the pathogenicity of _Arcobacter_ in male animals.

**MATERIALS AND METHODS**

**Experimental animals**

Thirty male five-month-old healthy albino rats ( _Rattus norvegicus_ ) weighing 200–250 g were acquired from the animal house unit of the college of Health Sciences, Ladoke Akintola University of Technology (LAUTECH), Osogbo, Nigeria. They were housed in transparent plastic cages 33 × 20.5 × 19 cm. The animals were fed on an antibiotics-free ration and given water _ad libitum_. They were housed five rats per cage and tagged before inoculation. The care and handling of the animals complied with the guidelines of the Ladoke Akintola University of Technology and of the National Institute of Health for the care of laboratory animals.

**Preparation of Arcobacter inoculums and procedure**

The _Arcobacter butzleri_ strain isolated from pigs in Nigeria, earlier confirmed by PCR and maintained in stock cultures at –25 °C glycerol _Arcobacter_ broths, was resuscitated in brain heart infusion agar supplemented with 5% of yeast and 7% of sheep blood and incubated at 35–37 °C in a microaerophilic atmosphere (1). Bacteria were collected at the exponential growth stage and diluted in 0.95 % normal saline. The suspension was later standardized by McFarlands Nephelometry to 10^8 CFU (colony-forming units) per ml. After the rats had been anaesthetized with inspired ether, 0.5 ml of _A. butzleri_ suspension containing 10^6 CFU was injected into the proximal right ductus deferens of the rats’ testes, and 0.5 ml of sterile normal saline solution was given to other sets of rats to serve as the control for the experiment. Five animals from each group were sacrificed on days 5, 10, 15, 20 and 30 of the experiment after bacterial inoculation, and organ specimens from the testes and epididymis sections of the rats were fixed by immersing in the Bouins fluid for 24 hours and later preserved in 10% formalin for 48 hours, embedded in paraffin (2–3 mm thick), stained with haematoxylin and eosin (H & E) and examined microscopically for histopathological changes. For sperm count and motility evaluation, several cuts on the testes were made, teased out into bits and thereafter suspended in 2 ml of Ham F solution. The latter was incubated for 10 minutes at 37 °C. Later, 50 µl were transferred with the aid of a sterile pipette into a haemocytometer chamber for phase contrast microscopy.

**RESULTS**

There were no visible gross lesions on the surface of the testes and epididymis. The germinal epithelium and basement membrane of testes in the control group were intact, showing tubules with active spermatogenesis (Fig. 1). The test group that received 10^6 CFU bacterial inoculums after day 5 to 30 had a varying degree of sperm count and motility post inoculation. After a single challenge, as the days passed by, there was a slight but not significant increase in the size of testes and the epididymis, but a significant reduction in spermatozoa count and motility (P < 0.05) in the test group was observed (Table). Degenerative changes in the test group consisted of a thickening of the basement membrane and absence of spermatozoa within the tubules (Fig. 2).
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**DISCUSSION**

Various pathologic concepts have evolved from experimental and clinical studies to elucidate the effects of bacteria on the testicular structure and function (24). *Arcobacter* has been implicated in cases of infertility in domestic animals (25). Results obtained in our study showed that *Arcobacter* produced testicular degeneration, a significant reduction in sperm count and motility. Testicular degeneration accompanied by a reduced sperm count and motility is shown in Table 1.

**Table. Changes of fertility indices in male albino rats with *Arcobacter* experimental infection**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum 0.5 ml</th>
<th>Sperm count</th>
<th>Sperm motility</th>
<th>Organ weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Testes epididymis</td>
</tr>
<tr>
<td>Control</td>
<td>Sterile saline solution</td>
<td>154 ± 1.14</td>
<td>&gt;70%</td>
<td>0.57 ± 0.03 0.17 ± 0.01</td>
</tr>
<tr>
<td>Test group (day 5)</td>
<td>10^8 cfu</td>
<td>144 ± 1.10</td>
<td>&gt;50%</td>
<td>0.59 ± 0.01 0.18 ± 0.01</td>
</tr>
<tr>
<td>Test group (day 10)</td>
<td>10^8 cfu</td>
<td>110 ± 1.11</td>
<td>&gt;40%</td>
<td>0.60 ± 0.03 0.20 ± 0.01</td>
</tr>
<tr>
<td>Test group (day 15)</td>
<td>10^8 cfu</td>
<td>100 ± 1.00</td>
<td>&gt;40%</td>
<td>0.61 ± 0.03 0.18 ± 0.00</td>
</tr>
<tr>
<td>Test group (day 20)</td>
<td>10^8 cfu</td>
<td>110 ± 1.11</td>
<td>&gt;50%</td>
<td>0.58 ± 0.03 0.17 ± 0.01</td>
</tr>
<tr>
<td>Test group (day 30)</td>
<td>10^8 cfu</td>
<td>120 ± 1.00</td>
<td>&gt;60%</td>
<td>0.58 ± 0.02 0.18 ± 0.00</td>
</tr>
</tbody>
</table>

The data are a mean of 5 replicates ± standard deviation. P < 0.05 significant.

Fig. 1. Normal testis shows tubules with active spermatogenesis. Control: (H & E × 100)

Fig. 2. Absence of spermatozoa in the tubules, marked degeneration and thickening of the basement membrane (H & E × 100)
motility in the test group supports the concept that
the inflammation which accompanies the concomitant dam-
age of the seminiferous tubules could result in infertility.
A slight insignificant post-infection increase in the size of
the testes and epididymus could be due to the infiltra-
tion of inflammatory cells and fluid which accompanies a
normal response to bacterial infection (26). Although the
behavioral sexual potential was not investigated in this
study, it is noteworthy that without therapeutic interven-
tion, sperm count, motility and organ size appeared to un-
dergo resolution. It could be suggested from this study that
the degenerative changes produced by Arcobacter appear
to be self-limiting. In conclusion, Arcobacters appears to
possess the capacity to induce testicular degeneration and
hence male infertility which could lead to herd infertility
in farm animals.

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**ARCObACTER BUTZLERI INFEKUOTŲ ALBINOSIŲ ŽIURKIŲ HISTOPATOLOGINIAI IR FUNKCINIAI SĖKLIDŽIŲ POKYČIAI**

**Santrauka**

**Įvadas.** *Arcobacter butzleri* infekcija yra plintanti enteropatogeninė zoonozę, susijusi su naminių gyvulų spontaniniais abortais, viduraviimu ir mastitais.

**Medžiaga ir metodai.** Trisdešimt sveikų albinosų patinų žiurkių buvo apkrėsti *Arcobacter butzleri* (10⁶ cfu/ml) infekcija, išskirta iš sveikų kiaulių išmatų, siekiant nustatyti *A. butzleri* infekcijos poveikį žiurkių sėklidžių histologijai ir spermatogenezei.

**Rezultatai.** *A. butzleri* sveikoms žiurkėms sukėlė sėklidžių degeneraciją, susijusią su spermatozoidų kiekiu ir judrumu.

**Išvados.** Šio tyrimo rezultatai leidžia teigti, kad *A. butzleri* sukėlia sėklidžių degeneraciją, stabdo spermatogenezę albinosėms žiurkėms, tačiau šios infekcijos poveikis nevaisingumui gyvulų pramonėje ir ekonominė nauda dar turi būti tiriama.

**Raktažodžiai:** *Arcobacter butzleri*, sėklidžių degeneracija, nevaisingumas, patogeniškumas, spermatozoidų kiekiš, albinosės žiurkės