The Effect of \textit{Clostridium difficile} Experimental Infection on the Health Status of Weaned Rabbits

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ABSTRACT

In this study, a trial to detect the effect of \textit{Clostridium difficile} (\textit{C. difficile}) experimental infection on the health status of weaned rabbits was carried out. Thirty, 5 week old weaned New Zealand rabbits were used. Rabbits were kept for a week under observation for adaptation and ensuring absence of any clinical signs, mortalities and anaerobic infections. Rabbits were randomly divided into 3 equal groups; 10 rabbits for each. Each rabbit in group (1) was subcutaneously (S/C) inoculated with 1 ml containing $1 \times 10^8$ colony forming unit (CFU) of \textit{C. difficile}, while each one in group (2) was orally inoculated with 2 ml ($3 \times 10^{10}$ CFU). Group (3) was kept as non-infected control one. During 3 weeks observation period; clinical signs, mortalities, gross lesions, histopathological changes and \textit{C. difficile} re-isolation were detected. The results indicated absence of clinical signs in \textit{C. difficile} S/C challenged rabbits, while signs of bloat and brownish diarrhea were observed in orally challenged ones. No mortalities were recorded in rabbits of both challenged groups. Lesions observed in sacrificed orally challenged rabbits were enlargement and congestion of liver and kidneys as well as mild degree of enteritis. The challenging \textit{C. difficile} was re-isolated from sacrificed rabbits at the end of the study. It could be concluded that \textit{C. difficile} is an organism of economic importance for newly weaned rabbits as it can badly effect on rabbits’ health status.

Key words: Rabbits, \textit{C. difficile}, Signs, Lesions, Isolation

Introduction

In the past 30 years, however, \textit{Clostridium difficile} (\textit{C. difficile}) has been implicated as the principal infectious cause of diarrhea in humans, and similar clinical conditions in a variety of other mammals (Songer, 1996 and Kelly and LaMont, 1998). The relevance of \textit{C. difficile} to disease in animals has also become more obvious but differs according to species, age, environmental density of spores, administration of antibiotics and possibly other factors (Keel and Songer, 2006). Lesions attributed to spontaneous or experimentally induced \textit{C. difficile} infection in rabbits were documented (Carman and Evans, 1984). Severe \textit{C. difficile} toxin-induced rabbit enteritis which characterized by exuberant intestinal tissue inflammation, epithelial disruption and diarrhea (Cirle \textit{et al.}, 2012).

So, this work was planned to study the effect of \textit{C. difficile} experimental infection on the health status of weaned rabbits through detection of the developed clinical signs, mortalities, gross lesions, histopathological changes in the affected tissues and \textit{C. difficile} re-isolation.

Materials And Methods

Rabbits:

Thirty, 5 week old, newly weaned New Zealand rabbits of mixed sex were obtained from a commercial farm and then housed in thoroughly cleaned and disinfected batteries. The rabbits received ration without feed additives. Feed and water were given to rabbits \textit{ad libitum}. The rabbits were kept for one week observation period for adaptation and for detection of any signs or mortality before experimental work. Rabbits were vaccinated by rabbit's viral hemorrhagic diseases vaccine at 7 weeks of age (1cm/ rabbit) and also received formalized polyvalent rabbit's pasteurellosis vaccine at 8 weeks old (1cm/ rabbit).
**Clostridium difficile strain:**

Identified field strain of *C. difficile* that isolated from apparently healthy, diseased and dead weaned rabbits were kindly obtained from Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

**Preparation of *C. difficile* inoculum used in experimental infection:**

Inoculum of *C. difficile* was prepared by plate count technique according to Mostafa, (1992) as follow:

For subcutaneous (S/C) route:
- Concentration of the inoculum was $1 \times 10^8$ colony forming unit (CFU).
- Dose of the inoculum was 1 ml/each rabbit.

For oral route:
- Concentration of the inoculum was $3 \times 10^{10}$ (CFU).
- Dose of the inoculum was 2 ml/each rabbit.

**Experimental design:**

Thirty, 5 week old, newly weaned New Zealand rabbits were used in experimental infection. Rectal swabs were collected from purchased rabbits at arrival as well as feed and water samples were examined to ensure their freedom of anaerobic infections. Thirty rabbits were randomly divided into 3 equal groups, 10 rabbits for each.

The experimental design including the groups, routes of inoculation and concentration and dose of inoculum are summarized in Table (1).

<table>
<thead>
<tr>
<th>Group Number</th>
<th>No. of Rabbits/group</th>
<th>Clostridium difficile challenge</th>
<th>Routes of inoculation</th>
<th>Concentration of inoculum</th>
<th>Dose of inoculum/rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>+</td>
<td>Subcutaneously</td>
<td>$1 \times 10^8$ CFU</td>
<td>1 ml</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>+</td>
<td>Orally</td>
<td>$3 \times 10^{10}$ CFU</td>
<td>2 ml</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Measured parameters:**

**Clinical signs and mortalities:**

Rabbits were kept for three weeks observation period post challenge. Clinical signs and mortalities were observed daily till the end of the study.

**Post-mortem lesions:**

Any dead rabbits during observation period were subjected to post-mortem examination for detection of lesions.

**Histopathological examination:**

Tissue specimens including liver, kidneys and small and large intestines were collected from sacrificed rabbits of all groups at the end of the work for histopathological examination (Banchroft *et al.*, 1996).

**Bacterial re-isolation:**

Rectal swabs from living rabbits as well as liver and intestinal samples from sacrificed rabbits were collected for *C. difficile* re-isolation (Smith and Holdman, 1968).

**Results And Discussion**

*Clostridium difficile* is a Gram-positive, anaerobic and spore-forming bacillus commonly associated with diarrhea and colitis in humans and other mammals (Songer, 1996).

This study was designed to study the effect of *C. difficile* experimental infection on the health status of weaned rabbits regarding clinical signs, mortalities, gross lesions, histopathological changes in the affected tissues and *C. difficile* re-isolation.
No clinical signs, mortalities or lesions were observed in non-infected control rabbits group along the whole experimental period. No clinical signs were seen in rabbits challenged with *C. difficile* after S/C inoculation, while signs of severe brownish diarrhea (Fig. 1) and bloat (Fig. 2) were detected in some animals of orally infected rabbits. Similarly, Prescott, (1977) and Keel and Songer, (2006) referred to the role of *C. difficile* in induction of enteritis in rabbits.

Rabbits challenged with *C. difficile* either in oral or S/C route showed no mortalities along the observation period. At the end of the study, *C. difficile* S/C challenged sacrificed rabbits revealed no gross lesions. However, orally challenged sacrificed ones showed enlargement and congestion of the liver (Fig. 3) and kidneys (Fig. 4), mild degree of enteritis (Fig. 5) with un-digested feed particles mixed with slimy exudates in the small intestine but the large intestine contained watery brownish contents (Fig. 6) with offensive odour. Comparable results were reported by Rehg and Shoung, (1981) and Mitchell *et al*., (1986) who considered *C. difficile* is a cause of cecitis in rabbits, also, Perkins *et al*., (1995) found that spontaneous *C. difficile* associated disease in rabbits is principally associated with lesions in the small intestine, especially the ileum, causing mucosal necrosis. Contrary results were obtained by Eglow *et al*., (1992) and Keel and Songer, (2006) who observed absence of both clinical signs and accordingly the lesions caused by *C. difficile* in neonate rabbits. Essential virulence factors of *C. difficile* are large exotoxins, toxin A (TcdA) does not affect ileal explants from 5-dayold rabbits, even at dosages that cause severe lesions in ileal explants from adults. A prominent hypothesis to explain the resistance of such neonates is that they lack the proper toxin receptors until later in life (Borriello and Wilcox, 1998). Binding of TcdA to ileal brush borders is decreased in neonatal rabbits, but maximal binding is observed in 90-day-old rabbits (Eglow *et al*., 1992).

The histopathological examination of scarified rabbits at the end of experimental trial revealed that there was no histopathological alteration observed in the tissue specimen collected from control group as there was normal histological structure of liver, kidney, small intestine and large intestine, Group of rabbits orally challenged with *C. difficile* showed congestion in the central vein associated with ballooning and degeneration in the hepatocytes (Fig. 7.A), vacuolization in the lining endothelium of the glomerular tuft associated with degeneration in the lining epithelium of the renal tubules (Fig. 7.B), fusion in the villi of small intestine with inflammatory cells infiltration in the lamina propria (Fig. 7.C) and massive number of inflammatory cells infiltration was detected in the lamina propria associated with oedema in the sub-mucosal layer of large intestine (Fig. 7.D). Group of rabbits exposed to *C. difficile* S/C experimental infection revealed dilatation in the portal vein associated with degenerative change in the hepatocytes (Fig. 8.A), vacuolization in the lining endothelium of the glomerular tuft associated with degeneration in the lining epithelium of the renal tubules (Fig. 8.B), diffuse goblet cells formation in the lining mucosal epithelial cells of small intestine associated with inflammatory cells infiltration in the lamina propria (Fig. 8.C) and massive number of inflammatory cells infiltration was detected in the lamina propria of large intestine (Fig. 8.D). Generally, in all *C.difficile* experimentally inoculated groups, the microscopical alterations in liver, kidneys and intestines are nearly similar to that recorded by Mitchell *et al*., (1986).

The results of *C. difficile* re-isolation from either rectal swabs or liver and intestinal samples showed no re-isolation of any *C. difficile* organisms from control non infected rabbits, while *C.difficile* was re-isolated from challenged groups, where it appeared on blood agar as glossy, grey and circular colonies with rough edges and no haemolysis (Fig. 9) and a characteristic farm yard smell odour.

**Conclusion:**

From the obtained abovementioned results, it could be concluded that *C. difficile* is an organism of economic importance for newly weaned rabbits as it can badly effect on rabbits’ health status. Further researches may be needed to explain the pathogenesis of *C. difficile* infections and the mechanisms of rabbit’s colonization as they are points of particular importance. Such information could improve animal welfare and livestock revenues.
Fig. 1: A rabbit orally infected with *C. difficile* with signs of severe brownish diarrhea.

Fig. 2: A rabbit orally infected with *C. difficile* with signs of bloat.

Fig. 3: A liver of rabbit orally infected with *C. difficile* showed enlargement and congestion.

Fig. 4: A kidneys of rabbit orally infected with *C. difficile* showed enlargement and congestion.

Fig. 5: Small intestine of rabbit orally infected with *C. difficile* showed enteritis.

Fig. 6: Large intestine of rabbit orally infected with *C. difficile* showed watery brownish contents.
Fig. 7: Histopathological findings of group of rabbits orally challenged with *C. difficile* sacrificed at the end of the experimental period, (A) liver showing congestion in the central vein (cv) with ballooning degeneration in the hepatocytes (d). H&E X64 (B) kidney showing vacuolization in the lining endothelium of the glomerular tuft (g) with tubular degeneration (d) H&E X80 (C) small intestine showing fusion of the villi with inflammatory cells infiltration (m) in the lamina propria H&E X40 (D) large intestine showing inflammatory cells infiltration in the lamina propria (m) with oedema in the submucosa (o) H&E X40.

Fig. 8: Histopathological findings of group of rabbits subcutaneously challenged with *C. difficile* at the end of the experimental period, (A) liver showing dilatation in the portal vein (pv) and degeneration in the hepatocytes (d) H&E X64 (B) kidney showing vacuolization in the lining endothelium of the glomerular tuft (g) with tubular degeneration (d) H&E X64 (C) small intestine showing goblet cells formation in the lining mucosal epithelium (g) with inflammatory cells infiltration (m) H&E X40 (D) large intestine showing massive number of inflammatory cells infiltration (m) in the lamina propria H&E X40.
Fig. 9: Colonies of *C. difficile* organism on blood agar shows glossy, grey and circular colonies with rough edges and no haemolysis.

References


