International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (6), pp. 001-007, June, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Escherichia coli O157:H7 EDL933 has a strong virulence to Bama miniature pigs by injection and fails to colonize to their gastrointestinal tracts

Honglei Ding[1,2,3], Rui Zhang[1], Kaiyun Liu[1], Linping Huang[1], Maochun Tian[4], Mingming Jiang[1], Quanming Zou[1] and Xuhu Mao[1]*

[1]Department of Clinical Microbiology and Immunology, Faculty of Medical Laboratory Science, Third Military Medical University and National Engineering Technological Research Center of Immunological Biologicals, Chongqing, China.
[2]Institute of Sericulture and System Biology, Southwest University, Chongqing, China.
[3]Chongqing Productivity Council, Chongqing, China.
[4]College of Animal Science and Technology, Southwest University, Chongqing, China.

Accepted 21 March, 2018

Detection of Shiga toxin-producing *Escherichia coli* O157:H7 from commercially grown pigs has been reported. Furthermore, the *E. coli* O157:H7 colonized model of pig has been established and *E. coli* O157:H7 could be transmitted from infected donor pigs to naïve pigs directly and indirectly. In the present study, we want to know whether any *E. coli* O157:H7 strain can colonize to the alimentary tract of pig and the virulence of *E. coli* O157:H7 to pig by injection. Bama miniature pig was infected with *E. coli* O157:H7 EDL933 strain orally, but the organism could not be recovered from the feces and did not cause any tissue damage. Nevertheless, this pathogen introduced serious clinical symptoms and pathological injuries by injection, especially the nervous system and the injected pig exhibited severe neurological symptoms, including synclonus tremens, ataxia, head-pressing and recumbency, etc. The pig did not excrete urine and feces and the abdomen became tympanous. These data suggested that only certain *E. coli* O157:H7 strains could colonize to the GIs of pigs involved mechanisms that related to various factors. However, the organism has strong virulence to pig by injection mode and it is a risky pathogen to human health.

Key words: *Escherichia coli* O157:H7, Bama miniature pig, colonization, inoculation, pathological injury.

INTRODUCTION

Infection of Shiga toxin -producing *Escherichia coli* (STEC) O157:H7 can lead to a spectrum of illnesses in human, including diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), which demonstrates as acute renal failure and may lead to death (Besser et al., 1993; Bruce et al., 2003; Rivas et al., 2006).

Most cases are thought to occur as a result of the ingestion of ground beef (Bell et al., 1994), unpasteurized milk (Solomakos et al., 2009) and vegetables (Besser et al., 1993), which are thought to have been contaminated with feces from infected cattle. Outbreaks and sporadic cases have also been linked to water, animal-to-person and person-to-person transmission (Swerdlow et al., 1992; Belongia et al., 1993; Shukla et al., 1995).

Cattle are considered to be the major reservoir of STEC and the prevalence of *E. coli* O157:H7 in cattle is range (Baker et al., 2007; Wang et al., 2008; Williams et al., 2008). *E. coli* O157:H7 has also been isolated from other ruminants, such as deer (García-Sánchez et al., 2007; Sánchez et al., 2009) and sheep (Kudva et al., 1996). *E. coli* O157:H7 has occasionally been isolated from non-ruminant animals, including poultry (Baschkier et al., 2009; Heuvelink et al., 1999), pigeons (Cízek et al., 2000; Kobayashi et al., 2002), wild birds (Wallace et al., 1997; Kobayashi et al., 2002) and raccoons (Hancock et al., 1998), but the bulk of the data suggests that the,

*Corresponding author. E-mail: maoxuhu@163.com, mxh95xy@mail.tmmu.com.cn, qmzou@mail.tmmu.com.cn Tel: 86-23-68752315. Fax: 86-23-68752315.

prevalence of STEC is greater in ruminants than in other animals.

Recent studies have demonstrated that conventional pigs were permissive host for *E. coli* O157:H7 (Booher et al., 2002; Cornick and Helgerson, 2004) and have established that *E. coli* O157:H7 could be shed by 3- month-old pigs for 2 months. Furthermore, these animals did not become clinically ill, and the duration of shedding in the feces was similar to that of ruminants experimentally infected with the same *E. coli* O157:H7 isolate (Booher et al., 2002) and could transmit this pathogen to their offspring. Meanwhile, some researches indicated that *E. coli* O157:H7 had been isolated from healthy pig in many countries (Heuvelink et al., 1999; Wang et al., 2008; Oporto et al., 2008).

Nevertheless, the prevalence of the organism in these studies was generally low, except for the result from Chile that the prevalence of *E. coli* O157:H7 was higher in pigs than in cattle, which suggested that pig might be an important source of this organism in some countries (Borie et al., 1997). Also, only one family outbreak has been specifically traced back to pork salami and the *E. coli* O157 isolated from the couple and the salami carried Shiga toxin 1 (stx1), Shiga toxin 2 (stx2) and *E. coli* attaching and effacing (eae) genes and shared the same PFGE (pulsed- field gel electrophoresis) pattern (Conedera et al., 2007) . To date, there is no explanation for the reason why the prevalence of *E. coli* O157:H7 in pig is generally lower than in ruminants. Also, no research about the consequence of pig infected this organism in its organs or abdomen, though these animals did not become clinically ill infected this pathogen orally.

We hypothesized that the gastrointestinal tract (GI) of pig did not facilitate the colonization of most *E. coli* O157:H7 strains and caused the low prevalence of *E. coli* O157:H7 in pig. In the study, *E. coli* O157:H7 EDL 933 was selected, which obtained from a patient whose symptom, characterized by severe cramped abdominal pain, initially watery diarrhea and grossly bloody diarrhea (Riley et al., 1983) and determined whether it was a suitable strain for the colonization to the GI of pig. Also, the virulence of *E. coli* O157:H7 to pig was detected by injection.

MATERIALS AND METHODS

E. coli O157:H7 strain

E. coli O157:H7 strain EDL933 (stx1, stx2, eaeA, ehxA, Tccp and espA positive) was originally isolated from patient with diarrhea (Riley et al., 1983) and was kindly provided by Prof. Huaiqi Jing (Chinese Center for Disease Control and Prevention). *E. coli* O157:H7 CYB42 was isolated from diary cattle in Chongqing (Wang et al., 2008). These bacterial inocula were grown as previously described with minor modification (Booher et al., 2002). Briefly, a single colony from strain was picked and cultured overnight in Luria-Bertani (LB) medium at 37°C with shaking (180 rpm) and the bacterial number was confirmed by direct plate counts. The inoculum was washed once with 0.1 M phosphate buffered saline (PBS) (pH 7.2) and adjusted to the appropriate concentration.

Animals and preparation

Bama miniature pigs were obtained from a commercial source at 1 month of age and housed in pens with cement floors at 2 pigs per pen under biohazard level 2 facilities. The pigs were acclimated to an antibioticfree feed (creep feed, Chongqing Zhengda Company, Chongqing) and water *ad libitum* for 2 weeks prior to inoculation. Fecal samples were collected from each animal once prior to inoculation and injection and screened with sorbitol-MacConkey agar supplemented with cefixime (2.5 $mg \cdot l^{-1}$) and potassium tellurite (0.05 mgl^{-1}) (CT- SMAC) and polymerase chain reaction (PCR) to ensure that the pigs were not naturally colonized by *E. coli* O157:H7.

Inoculation of *E. coli* O157:H7

18 pigs were used in inoculation and were separated into 3 groups, of which 16 were inoculated with the EDL933 and 2 were negative controls. After a 2 week acclimation period, groups 1 and 2 of each 8 pigs were inoculated with 1.0×10^8 and 1.0×10^{12} clonal formation unit (CFU) of *E. coli* O157:H7 EDL933 by adding the organism to a small amount of food placed in individual pans, respectively. On the 17th day after fed EDL933 bacteria, groups 1 and 2 of each 4 pigs, selected randomly, were inoculated with 1.0×10^8 and 1.0×10^{12} CFU of *E. coli* O157:H7 CYB42. Group 3 of 2 pigs were inoculated with 10 ml 0.1 M PBS (pH 7.2). Pigs were observed until the inoculum was consumed.

Injection of *E. coli* O157:H7 lysate

18 pigs were used in injection and were also separated into 3 groups. After a 2 week acclimation period, group 1 of 8 pigs were injected with 1.0×10^8 CFU of *E. coli* O157:H7 strain EDL933 lysate intramuscularly; group 2 of 8 pigs were injected with 1.0×10^8 CFU *E. coli* O157:H7 EDL933 lysate intraperitoneally; and group 3 of 2 pigs were injected with 10 ml 0.1 M PBS (pH 7.2) intramuscularly.

Fecal sampling

Individual fecal samples from inoculated pigs were collected on days 2, 3, 4 and at 2 weeks post inoculation (pi) (days 14, 15, 16) of EDL933 and CYB42, respectively, and from injected pigs were collected on days 2, 3, 4 (Booher et al., 2002). Fecal samples were cultured as previously described (Cornick and Helgerson, 2004). Briefly, 5 g samples were added to 20ml of PBS (pH 7.2) and mixed in a Stomacher blender, and then serial 10 fold dilutions were made to use PBS (pH 7.2).

Samples were directly inoculated in triplicate into selective media CT-SMAC. Enrichment cultures (10 g of feces in 100 ml LB plus 0.02% bile salts) were incubated overnight at 37°C, concentrated using immunomagnetic beads (Dynabeads; Dynal, Oslo, Norway), and plated into the selective medium described above. The sensitivity of the direct plating method was 50 CFU/g. Colonies recovered on selective medium were confirmed as *E. coli* O157:H7 by using a commercial latex agglutination kit specific for the O157 lipopolysaccharide and PCR for *rfbE* (Wang et al., 2008).

Necropsy

The animals monitored closely to ensure their welfare after they were injected. Both control and *E. coli* EDL933 injected animals were euthanized and necropsied at the 4th day when they behave severe clinical signs. Meanwhile, after 2 weeks pi, control, *E. coli* EDL933 (8 pigs) and *E. coli* CYB42 (8 pigs) inoculated animals orally were euthanized and necropsied. Pigs were sedated with an injectable anesthetic (tiletamine HCl and zolazepaam HCl) and then euthanized with an intravenous overdose of sodium barbiturate (390 mg pentobarbital sodium and 50 mg phenytoin sodium/5 kg of body weight) (Booher et al., 2002).

The following types of tissue (approximately 5 cm length or 5 cm square) were collected from pigs: stomach, jejunum, ileum, distal colon, cecum, rectum, epencephalon, cerebrum, kidney, liver, and spleen. The sections of tissues were collected in neutral buffered formalin for histopathology, processed, and stained with hematoxylin and eosin (H and E). 5 g of rectal contents were also collected and cultured by using direct plating and enrichment broth as described above.

Western blotting

Serum samples were collected from both control and *E. coli* inoculated pigs prior to inoculation and at necropsy and detected stx2, intimin and espA neutralizing antibodies. The stx2, intimin and espA recombinant purified proteins were prepared in our lab (Gu et al., 2009; Ma et al., 2008). SDS-PAGE and western blotting were performed as described previously (Cendron et al., 2009). For visualization of proteins after SDS-PAGE, gels were stained with Coomassie brilliant blue R250.

For the development of immunoblots, PVDF filters were blocked with blocking buffer (Beyotime) and incubated with the respective antisera at a dilution of 1:1000. The membrane was washed 6 times with TBS containing 0.1% Tween-20 (TTBS) (pH 7.5). Horseradish peroxidaseconjugated anti-rabbit IgG was used at a dilution of 1:10 000 to visualize bound antibody.

RESULTS

Clinical response and bacterial culture of inoculated pigs

None of the animals developed signs of intestinal or systemic disease following inoculation with the *E. coli* inoculum from the 2 groups that were inoculated the strain orally. *E. coli* O157:H7 was not recovered from any of the 16 pigs during the initial period or at 2 weeks after inoculation with 1.0×10^8 and 1.0×10^{12} CFU. Fecal samples from the rectal-anal junction were also collected from these 16 pigs and no target organism was discovered.

Then, groups 1 and 2 of each 4 pigs were inoculated with 1.0×10^8 and 1.0×10^{12} CFU of *E. coli* O157:H7 CYB42. However, *E. coli* O157:H7 CYB42 was still not recovered from the 8 inoculated pigs. No *E. coli* O157:H7 was investigated from any of the two control animals at any time during the experiment, and no symptom displayed.

Clinical observation and bacterial culture of injected pigs

Piglets challenged intramuscularly and intraperitoneally with EDL933 typically developed neurological signs within 36 and 48 h, respectively, including anorexia, depression and paralysis of the hind limbs like goggy sitting. However, the feces are dry. 48 to 72 h following injection with *E. coli* O157:H7 strain, the piglets lay on one side, and went on to manifest severe neurological symptoms of synclonus tremens, ataxia, head-pressing and recumbency. Simultaneously, the mouth was slightly open and jerked violently and spasmodically and the 2 fore limbs stroked like swimming. The pig did not excrete urine and feces and the abdomen became tympanous.

When the anocelia was opened, a little of serous fluid leaked from the abdominal subcutaneous tissue, and the livers and spleen were adhered to the peritoneum. The intestinal wall was thinner than the normal one. There was too much urine in the bladder, which caused the tympanous abdomen. The blood became thick and reddish black with slow bloodstream.

No stx2, intimin and espA neutralizing antibodies were detected in both *E. coli* O157:H7-infected and *E. coli* O157:H7-injected pigs.

Histological studies on *E. coli* O157:H7-infected and *E. coli* O157:H7-injected pigs

We also studied the effect of *E. coli* O157:H7 injection on their main target organs, including stomach, intestine, epencephalon, cerebrum, kidney, liver and spleen by examining H and E stained-sections. The main pathological damage was observed at cerebrum (Figure 1), epencephalon (Figure 2) and large intestine (Figure 3). The nerve cells of cerebrum became spindle-shaped and hydropic, accompanying coagulation necrosis and the nucleus was pycnotic. In addition, blood capillary was atresic, and perivascular space was broadened.

Examination at epencephalon revealed the obvious reduction of cells in tunicae granulosa and neuropile porous. The members of the research group could also detect karyopycnosis of purkinje cell. Histological examination of ceca, recta of *E. coli* O157:H7 injected pigs showed multi- focal areas of villous or surface epithelium degeneration, necrosis, or shedding, submucous membranous hydropsia, and extensive inflammatory cell infiltration, mainly the lymphocyte in proper coat. Surprisingly, the small intestine and kidney (Figure 4) only showed slightly pathological change with mild cell trauma. For instance, some glomeruli of kidney increased in volume, and the capsular space became narrow.

Proximal convoluted tubule cells exhibited denaturation and necrosis. Meanwhile, the blood capillary was enlarged. Conversely, *E. coli* O157:H7-infected group of pigs did show any pathology in their tissues. There were no abnormalities in the control animals as well.

DISCUSSION

During the experiment, it was found that EDL933 could not infect Bama miniature pigs orally, though the

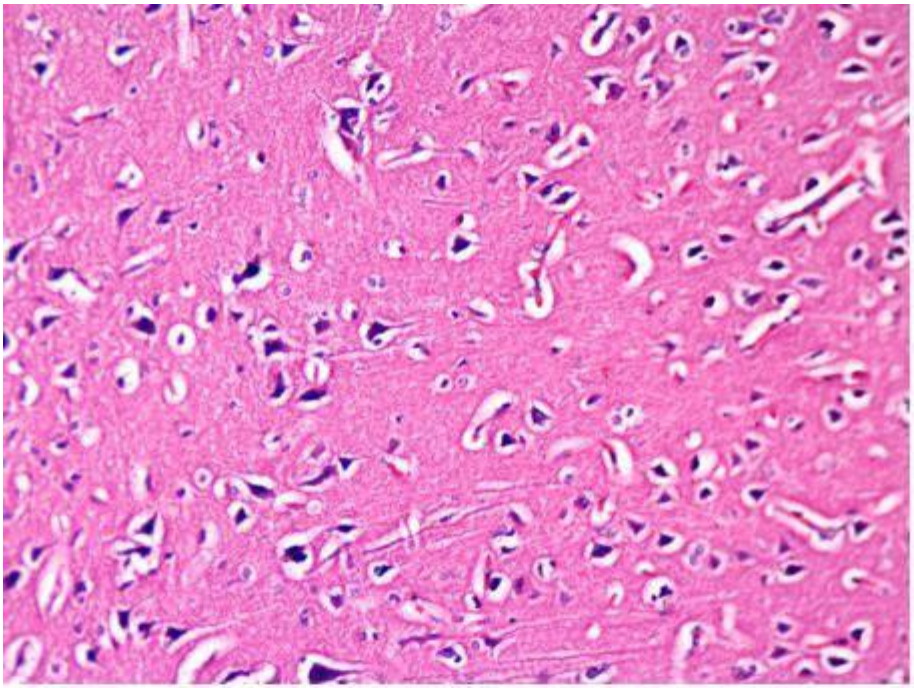

Figure 1. Cerebrum injury by injection of *E. coli* O157:H7 EDL933 (H&E 200×). The structure of cerebrum was loose, and the nerve cells became shrinked and triangular, with cytoplasmic basophilia, nucleus anachromasis, and entoblast unconspicuousness. In addtion, blood capillary was atresic, and perivascular space was broadened.

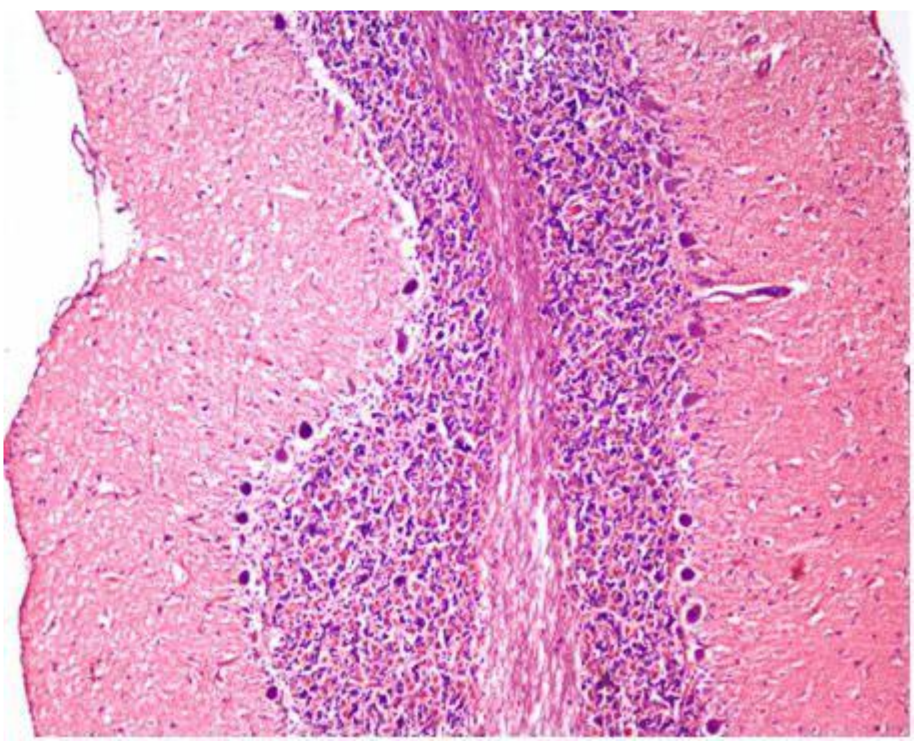

Figure 2. Epencephalon injury by injection of *E. coli* O157:H7 EDL933 (H&E 100×). Examination at epencephalon revealed the organization structure was loose and the nerve cell population in tunicae granulosa was obvious reduced. The size of purkinje cell could also be detect to be reduced, with cytoplasmic basophilia and nucleus anachromasis.

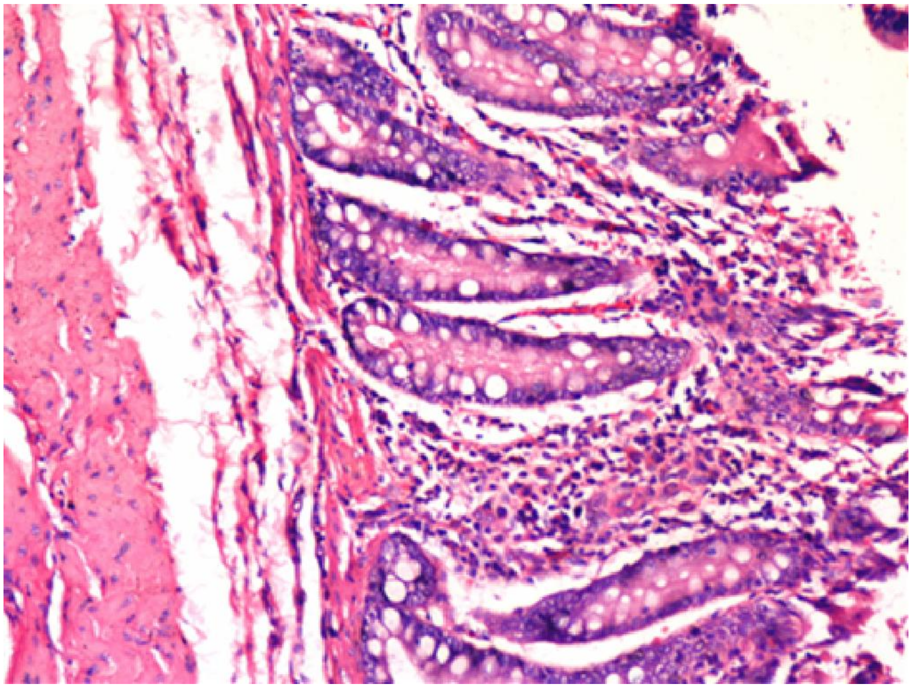

Figure 3. Rectum injury by injection of *E. coli* O157:H7 EDL933 (H&E 100×). Rectum showed intestinal epithelial cells necrosis, shedding, extensive inflammatory cell infiltration, loose organization structure in membrana propria and smooth muscle fiber tympanites.

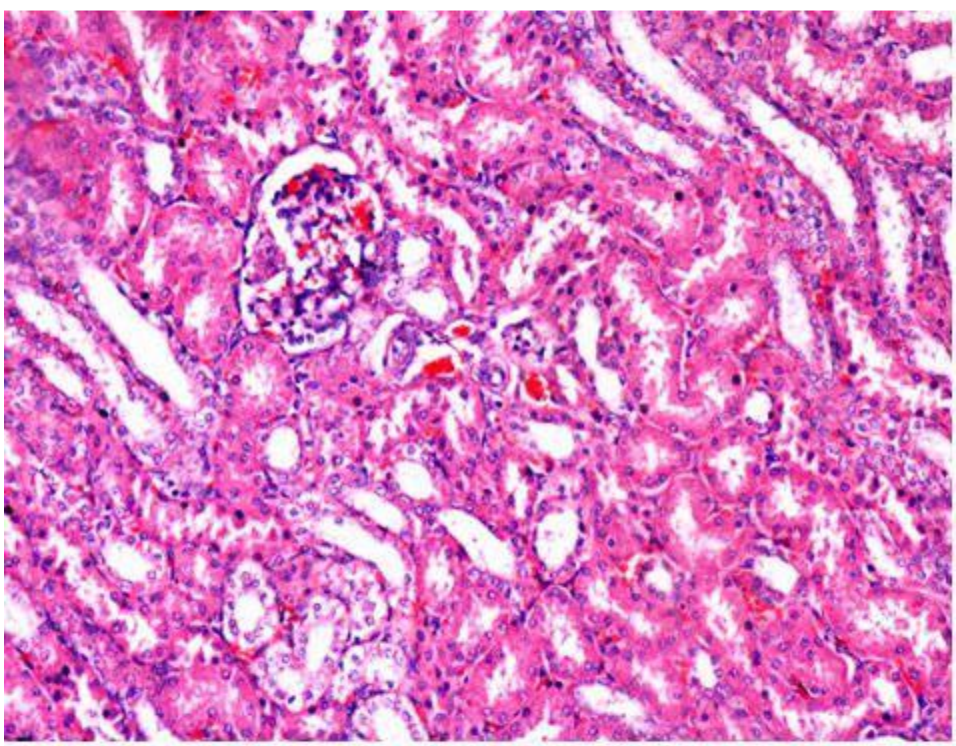

Figure 4. Kidney injury by injection of *E. coli* O157:H7 EDL933 (H&E 200×). Glomerular capillary was enlarged, and the cell population was increased. Glomeruli of kidney were increased in volume, accompanying narrow capsular space. Renal tubular epithelium exhibited denaturation and necrosis. Mesangiocapillary was enlarged and filled with akaryocytes.

infectious dose is high. In order to further confirm this outcome, the researchers chose *E. coli* O157:H7 CYB42 that was isolated from diary cattle in Chongqing to reinoculate the EDL933-feeded pigs. But, the result was negative as previously. It demonstrated, to some extent, that some pig individuals were resistant to colonization and/or some *E. coli* O157:H7 strains could not colonize to the intestinal epithelium of pig. Some researches indicated that many bacterial factors contributed to the colonization.

Some research data demonstrated that the flagellum of *E. coli* O157:H7, but not intimin, was important for persistence in poultry (Best et al., 2005), whereas intimin (Woodward et al., 2003) but not the flagellum was important in conventionally weaned lambs. This suggests that the function of both surface arrayed structures may be host dependent and may be linked to the availability of specific host-cell receptors. Best et al suggested that the flagellum and intimin of a *stx* -negative *E. coli* O157:H7 isolate had little or no role to play in colonization of 14 week-old conventionally reared pigs (Best et al., 2006).

However, other reported *E. coli* O157:H7 virulence factors, such as long polar fimbriae, did contribute to the persistence in pig animal infection model (Jordanet et al., 2004), and might have contributed to the persistent infection of pigs noted for the intimin and flagella deficient mutants reported. The strain, 86 - 24, which was isolated from an outbreak of human disease and caused attaching and effacing lesion, was used in most animal experiments and considered as a well established *E. coli* O157:H7 infected pathogen. The EDL933 strain, but not 86 - 24, may be absent from the essential factors, which were necessary for the long-term colonization of the pigs' intestinal tracts. This could partially explain why EDL933 could not adhere to Bama miniature pigs' GI. The genetic difference of the 2 strains for colonization in animals needs further comprehensive researches.

In this experiment, we also attempted to establish a Bama miniature pig model infected steadily with *E. coli* O157: H7, for the small weight of the Bama miniature pig of which the figure is thinner than susscrota domestica's. Nevertheless, the EDL933 strain could not colonize to the GIs of Bama miniature pigs. In the next plan, the research group will try to inoculate and acclimate EDL933 strain into Bama miniature pig through certain methods, such as serial passages in vivo, for procuring an adaptive colonization of *E. coli* O157:H7 strain.

To identify whether *E. coli* O157:H7 was pathogenic to pigs, nonproliferative EDL933 lysate were injected to Bama miniature pigs intramuscularly and intraperitoneally. Interestingly, the infected pigs exhibited identical clinical symptom, especial the nervous syndrome. However, the injected pigs did not manifest diarrhea, which was consistent to the histological findings. Serious intestinal microvillus damage may be an indispensable process caused through intestinal infection of *E. coli* O157:H7. Therefore, *E. coli* O157:H7 will be pathogenic when it

enters the organa parenchymatosums or abdomens of pigs

Conclusion

Collectively, only certain *E. coli* O157:H7 strains could colonize to the GIs of pigs. It explained the low prevalence of *E. coli* O157:H7 in pigs partially. However, this organism has strong virulence to pig by injection mode, and it is a risky pathogen to human health.

ACKNOWLEDGEMENTS

This work was supported by the 863 high-tech project of China (2006AA02Z443) and the national science foundation of China (30970116).

REFERENCES

Baker DR, Moxley RA, Steele MB, Lejeune JT, Christopher-Hennings J, Chen DG, Hardwidge PR, Francis DH (2007). Differences in virulence among *Escherichia coli* O157:H7 strains isolated from humans during disease outbreaks and from healthy cattle. Appl. Environ. Microbiol., 73: 7338-7346.

Bell BP, Goldoft M, Griffin PM, Davis M, Gordon DC, Tarr PL, Bartleson CA, Lewis JH, Barrett TJ, Well JG (1994). A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhoea and haemolytic uraemic syndrome from hamburgers. The Washington experience. J. Am. Vet. Med. Assoc., 272: 1349-1353.

Belongia EA, Osterholm MT, Soler JT, Ammend DA, Bruan JE, MacDonald KL (1993). Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. J. Am. Med. Assoc., 269: 883-888.

Besser RE, Lett SM, Weber JT, Doyle MP, Barrett TJ, Wells JG, Griffin PM (1993). An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. J. Am. Med. Assoc., 269: 2217–2220.

Best A, La Ragione M, Clifford D, Cooley WA, Sayers AR, Woodward MJ (2006). A comparison of Shiga-toxin negative *Escherichia coli* O157 aflagellate and intimin deficient mutants in porcine *in vitro* and *in vivo* models of infection. Vet. Microbiol., 113: 63-72.

Best A, La Ragione RM, Sayers AR, Woodward MJ (2005). Role for flagella but not intimin in the persistent infection of the gastrointestinal tissues of specific-pathogen-free chicks by Shiga toxin-negative *Escherichia coli* O157:H7. Infect. Immun., 73: 1836-1846.

Booher SL, Cornick NA, Moon HW (2002). Persistence of *Escherichia coli* O157:H7 in experimentally infected swine. Vet. Microbiol., 89: 69-81.

Borie C, Monreal Z, Guerrero P, Sanchez ML, Martinez J, Arellano C, Prado V (1997). Prevalencia y caracterizacion de *Escherichia coli* enterohemorragica aisladas de bovinos y cerdos sanos faenados en Santiago, Chile Arch. Med. Vet., 29: 205-212.

Bruce MG, Curtis MB, Payne MM, Gautom RK, Thompson EC, Bennett AL, Kobayashi JM (2003). Lake- associated outbreak of *Escherichia coli* O157:H7 in Clark county, Washington, August 1999. Arch. Pediat. Adol. Med., 157: 1016-1021.

Cendron L, Couturier M, Angelini A, Barison N, Stein M, Zanotti G (2009). The Helicobacter pylori cagD (HP0545, cag24) protein is essential for cagA translocation and maximal induction of interleukin-8 secretion. J. Mol. Biol., 386: 204-217.

Cízek A, Literák I, Scheer P (2000). Survival of *Escherichia coli* O157 in faeces of experimentally infected rats and domestic pigeons. Lett. Appl. Microbiol., 31: 349-352.

Conedera G, Mattiazzi E, Russo F, Chiesa E, Scorzato I, Grandesso S, Bessegato A, Fioravanti A, Caprioli A (2007). A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami. Epidemiol. Infect., 135: 311-314.

Cornick NA, Helgerson AF (2004). Transmission and infectious dose of *Escherichia coli* O157:H7 in swine. Appl. Environ. Microbiol., 70: 5331-5335.

García-Sánchez A, Sánchez S, Rubio R, Pereira G, Alonso JM, Hermoso de Mendoza J, Rey J (2007). Presence of Shiga toxin-producing *E. coli* O157:H7 in a survey of wild artiodactyls. Vet. Microbiol., 121: 373-377.

Gu J, Liu Y, Yu S, Wang H, Wang Q, Yi Y, Zhu F, Yu X, Zou Q, Mao X (2009). Enterohemorrhagic *Escherichia coli* trivalent recombinant vaccine containing EspA, intimin and Stx2 induces strong humoral immune response and confers protection in mice. Microbes. Infect., 11: 835-841.

Hancock DD, Besser TE, Rice DH, Ebel ED, Herriot DE, Carpenter LV (1998). Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. Prev. Vet. Med., 35: 11-19.

Heuvelink AE, Zwartkruis-Nahuis JTM, van den Biggelaar FLAM, van Leeuwen WJ, de Boer E (1999). Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. Int. J. Food Microbiol., 52: 67-75.

Jordan DM, Cornick N, Torres AG, Dean-Nystrom EA, Kaper JB, Moon HW (2004). Long polar fimbriae contribute to colonization by *Escherichia coli* O157:H7 *in vivo*. Infect. Immun., 72: 6168-6171.

Kobayashi H, Pohjanvirta T, Pelkonen S (2002). Prevalence and characteristics of intimin- and Shiga toxin-producing *Escherichia coli* from gulls, pigeons and broilers in Finland. J. Vet. Med. Sci., 64: 1071-1073.

Kudva IT, Hatfield PG, Hovde CJ (1996). *Escherichia coli* O157:H7 in microbial flora of sheep. J. Clin. Microbiol., 34: 431-433.

Ma Y, Mao X, Li J, Li H, Feng Y, Chen H, Luo P, Gu J, Yu S, Zeng H, Guo G, Yang K, Zou Q (2008). Engineering an anti-Stx2 antibody to control severe infections of EHEC O157:H7. Immunol. Lett., 121: 110-115.

Oporto B, Esteban JI, Aduriz G, Juste RA, Hurtado A (2008). *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in Northern Spain. Zoonoses. Public. Heal., 55: 73-81.

Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New. Engl. J. Med., 308: 681-685.

Rivas M, Miliwebsky E, Chinen I, Roldan CD, Balbi L, Garcia B, Fiorilli G, Sosa Estani S, Kincaid J, Rangel J, Griffin PM (2006). Characterization and epidemiologic subtyping of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic uremic syndrome and diarrhea cases in Argentina. Foodborne. Pathogen Dis., 3: 88-96.

Sánchez S, Martínez R, Rey J, García A, Blanco J, Blanco M, Blanco JE, Mora A, Herrera-León S, Echeita A, Alonso JM (2009). Pheno-genotypic characterisation of *Escherichia coli* O157:H7 isolates from domestic and wild ruminants. Vet. Microbiol., 142: 445-449.

Shukla R, Slack R, George A, Cheasty T, Rowe B, Scutter J (1995). *Escherichia coli* O157 infection associated with a farm visitor centre. Commun. Dis. Rep., 5: 86-90.

Solomakos N, Govaris A, Angelidis AS, Pournaras S, Burriel AR, Kritas SK, Papageorgiou DK (2009). Occurrence, virulence genes and antibiotic resistance of *Escherichia coli* O157 isolated from raw bovine, caprine and ovine milk in Greece. Food Microbiol., 26: 865-871.

Swerdlow DL, Woodruff BA, Brady RC, Griffin PM, Tippen S, Donnell HD.Jr, Geldreich E, Payne BJ, Meyer A Jr, Wells JG, Greene KD, Bright M, Bean NH, Blake PA (1992). A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Int. Med., 117: 812-819.

Wallace JS, Cheasty T, Jones K (1997). Isolation of vero cytotoxin-producing *Escherichia coli* O157 from wild bird. J. Appl. Microbiol., 82: 399-404.

Wang H, Mao X, Ding H, Zou Q, Peng X (2008). Epidemiological survey on *Escherichia coli* O157 in Chongqing and Three-Gorge Reservoir Areas of China. Vet. Res. Commun., 32: 449-461.

Williams AP, Gordon H, Jones DL, Strachan NJ, Avery LM, Killham K (2008). Leaching of bioluminescent *Escherichia coli* O157:H7 from sheep and cattle faeces during simulated rainstorm events. J. Appl. Microbiol., 105: 1452-1460.

Woodward MJ, Best A, Sprigings KA, Pearson GR, Skuse AM, Wales A, Hayes CM, Roe JM, Low JC, La Ragione RM (2003). Non-toxigenic *Escherichia coli* O157:H7 strain NCTC12900 causes attaching-effacing lesions and eae-dependent persistence in weaned sheep. Int. J. Med. Microbiol., 293: 299-308.