Abstract: Introduction and objective: Vibrio cholera, the human intestinal pathogen responsible for the diarrheal disease cholera, elaborates a large number of proteins, including several virulence factors. The cholera enterotoxin (CT) has been considered a major virulence factor of V. cholera. V.cholerae produces other putative toxins such as zonula occludens toxin (Zot) and accessory cholera enterotoxin (Ace). Zot has the ability to reversibly alter intestinal epithelial tight junctions, allowing the passage of macromolecules through the mucosal barrier. zot causes fluid accumulation in ligated rabbit ileal loops and cause mild diarrhea.

Materials and methods: In the current study, gene coding for the Zot toxin was amplified from V. cholerae isolate 62013 and producing a single band of 1200bp. The PCR product containing the Zot gene was cloned in pET28a expression vector .The recombinant zot gene was transformed into E. coli (DH5 α ) strain and then retransformed into E. coli (BL21) strain for expression of protein .Zot gene was induced with IPTG and affinity-purified by Ni2+-Sepharose resin. The recombinant zot protein was reacted with rabbit anti-Vibrio cholerae polyclonal antibody in western-blot analysis and rabbit ileal loop experiment was conducted.

Results : In our study, Zot was present as a band of approximately 45 kDa and 10-30 kDa. The protein was detected by Western blot. The present study is concerned with permeability changes after introduction of Zot protein in to loops of rabbit small intestine.

Conclusion: Our results confirmed that a prokaryotic expression system for zot protein was successfully constructed.

vibrio cholerae, cloning, expression, purification, zot., ileal loop
Presentation: Poster