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Molecular Significance of *lon* and *cpxR* Genes in the Pathogenicity of *Salmonella*

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Abstract

The important foodborne zoonotic pathogen *Salmonella* causes gastroenteritis. The dynamics of host-pathogen *Salmonella* interaction and infection might enhance the development of novel targeted preventative measures and drug regimens. The *lon* and *cpxR* are virulence associated genes, which have an important role in the *Salmonella* pathogenesis. However, the deletions of *lon* and *cpxR*lead to the construction of genetically engineered live *Salmonella* vaccine candidate. In this review, *lon* and *cpxR* genes are focused for their involvement in Salmonella pathogenesis. Furthermore, the importance of these genes was briefly emphasized during the construction of *Salmonella* vaccine candidate.

Keywords

***Salmonella*, *lon* and *cpxR*, Pathogenesis**

1. Introduction

Worldwide, salmonellosis is a major public health concern, which frequently causes gastroenteritis and zoonotic infections [1] [2]. In the United States, *Salmonella* spp. lead approximately 1.2 million human illnesses annually [3]. These infections are mainly acquired by exposure of contaminated food or infected animals [3] [4]. An initial step in the *Salmonella* pathogenesis is bacterial penetration of the intestinal epithelium. Penetration requires the expression of invasion genes, which are generally found in *Salmonella* pathogenicity island 1 (SPI1) [5]. SPI1 invasion genes encode a bacterial type III secretion apparatus and several effectors, which are important for interaction with eukaryotic proteins in pathogenesis [6] [7].

The understanding of within-host population dynamics of *Salmonella* infections is important for allowing

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delivery of targeted interventions. Among the strategies that have been used to control *Salmonella*, vaccination represents one of the most suitable strategies [8]. An understanding of within-host dynamics of *Salmonella enterica* interactions with eukaryotic cells could shape the development of vaccines. Comparative analysis of live and killed vaccines revealed that killed vaccines were unable to afford desired protection, while the suitable live vaccines were efficient in protection [9]. But, the potential for virulence reversal through horizontal gene transfer remains an important concern for live vaccines [10]. In *Salmonella*, significant involvement of *lon* and *cpxR* genes in the pathogenic mechanisms has been reported in the studies [11]-[15].

This review focuses on the importance of *lon* and *cpxR* genes in the *Salmonella* pathogenesis. In addition, the possibility of utilizing *lon* and *cpxR* genes for the construction of live vaccines was proposed due to their considerable involvement in pathogenic mechanisms [13]-[15].

2. *lon* Gene

Lon protease is a cytoplasmic protein in prokaryotes and a mitochondrial matrix protein in eukaryotes [16]. Lon is a member of four families of ATP-dependent proteases—including the Clp family (ClpAP and ClpXP), HslVU, and FtsH—which have been well characterized in bacteria [17]-[19]. Lon has four identical 87-kDa subunits, each consisting of a highly charged N-terminal domain, a centrally located ATP binding domain, and a proteolytically active C-terminal domain [20] [21]. Lon has been known as a powerful negative regulator for the expression of invasion genes encoded on *Salmonella* pathogenicity island 1 (SPI-1) through degradation of HilC and HilD. In addition, the invasive phenotype of *Salmonella* is negatively regulated by the ATP-dependent Lon protease, which is known to be a major contributor to proteolysis in *Escherichia coli*. Lon protein negatively regulates the ability of the bacterium to invade epithelial cells. It also affects macrophage survival, and is essential to cause systemic infection by *Salmonella* [5] [11] [22]. Lonis an evolutionarily conserved stress protein induced by multiple stressors. It assists to remove damaged and abnormal proteins during stress, and contributes to the cell division, cell morphology and DNA maintenance [5] [11] [23]-[26]. In addition, Lon participates in controlling multiple pathways: post-translational quality control [27], capsule synthesis through degradation of RcsA, which is a transcriptional activator of the biosynthetic genes [28], sporulation [29], cell cycle progression [30], lateral flagellar biosynthesis [31], negative regulation of type III secreted protein [32], ribosomal protein degradation after amino acid starvation [33], antitoxin protein degradation in toxin-antitoxin systems [34], bacterial fimbria and extra-cellular polysaccharide production [15].

3. *cpxR* Gene

The cell envelope of Gram-negative bacteria is composed of the inner membrane, the periplasmic space and the outer membrane. It is also exposed by flagella, porins, secretion systems and adhesions [35]. Different signal transduction systems permit *Salmonellae* to perceive alterations in the external environment or damage to their cellular components. After these alterations, physiology of *Salmonella* undergoes several changes in order to prolong survival. The response to alterations in the cell envelope is regulated by at least three extra cytoplasmic stress response (ESR) pathways in *Salmonella* spp., including the alternative sigma factor σ^E (RpoE) [36] [37], the two-component regulator CpxAR [38], and the two-component regulator BaeSR [39] [40]. CpxA/CpxR is two component (a sensor kinase/a response regulator) signal transduction pathway. CpxA (Sensor Kinase) is found in the cytoplasmic membrane, where it senses diverse signals, including alkaline pH, altered membrane lipid composition, interaction with hydrophobic surfaces, and misfolded pilin subunits. Subsequently, CpxAautophosphorylates and donates its phosphoryl group to activate CpxR, (Response Regulator). CpxR composed of an N-terminal receiver domain (REC) with an aspartate (D51) at the site of phosphorylation, and a C-terminal effector domain, which mediates the output response as a transcriptional regulator of target genes [41]. Interestingly, the balance between phosphorylated and dephosphorylated CpxR is crucial for the initiation and durability of a specific genetic response to the external stimulus [42] [43]. CpxAR also directly and indirectly inhibits the formation of the P pili [44]. CpxAR also governs the protein expressions such as DsbA and PpiA, which help in pilin assembly in the periplasm. CpxAR could be associated with negatively regulation of the expression of curli in *Salmonella*. Activated CpxR regulates part of the envelope stress response system, pilus assembly, type III secretion, motility and chemotaxis, adherence, and biofilm development. So, CpxR can be related to both adhesion and invasion of epithelial cells [45].

4. Genetically Constructed Vaccine Candidate after *lon* and *cpxR* Gene Deletion

After deletion of *lon* gene, the increased invasiveness can result from the accumulation of HilC and HilD, leading to overexpression of the SPI-1 genes, which are important for infective *Salmonella* to cross the small intestinal barrier [22]. A *lon* mutant can efficiently invade cultured epithelial cells, and enhanced production and secretion of three identified SPI1 proteins, SipA, SipC, and SipD. The expression of SPI1 proteins is also regulated in response to several environmental conditions. The disruption of the *lon g*ene can affect its replication in the host cell and its capability to cause overwhelming systemic disease [11]. The *lon* mutant can reach extraintestinal sites but unable to proliferate efficiently within the spleen of mice. Thus, Lon protease is essentially involved in the lethal systemic infection with *Salmonella* in mice. However, the *lon* mutant can not survive and proliferate within macrophage cells, suggesting that the Lon protease of *Salmonella* is involved in the withstanding of the killing mechanism of macrophage and in growth intracellularly. The reduced capability of the *lon* mutant to survive and grow in macrophage could be due to the enhanced susceptibility to the oxidative killing mechanism associated with respiratory burst and the low phagosomal pH. The overexpression of SPI1 genes by Londepletion leads rapid and massive macrophage apoptosis through a mechanism including caspase-1 and -3 [46]. In addition, CpxR mutant can develop protection against exposure to alkaline pH 8.0 during growth in broth. However, the nature of this process remains unknown [45]. The *cpxR* mutants were more efficiently internalized in the eukaryotic cells than the wild type strain [47].

After deletions of *lon* and *cpxR* genes, the mutants showed more fimbria and capsular productions than those of the wild type *Salmonella* [13]-[15]. Thus, the mutant strains constructed with deletions of *lon* and *cpxR* showed increase capability for adhesion or invasion, but decreased survival, replication and systemic infection in the host cell, resulting in easy eradication from host cells without causing side effects. In addition, the chances of reversion to the wild-type phenotype are less because of the complete deletion of two virulence-associated genes, *lon* and *cpxR*. Since, capsular polysaccharides are major antigenic components, which can induce strong immune responses for protection against pathogens [13]. In this way, the *lon* and *cpxR* gene deleted *Salmonella* mutant showed effective vaccine candidate against *Salmonella* serovars [13]-[15].

5. Significance of Developed Vaccine Candidate

The high productions of fimbria and capsular polysaccharides by lon and cpxR deleted *Salmonella* mutants showed elevated immune responses, which can subsequently protect against *Salmonella* infections [13]-[15]. In addition, the developed mutant vaccine candidate is used for delivery of heat-labile enterotoxin B subunit protein (LTB) of *E. coli* as an adjuvant to enhance immune responses and protection efficacy against Salmonellosis [48]-[50]. Development of a reliable vaccine is critical, as salmonellosis has global effects on human health. The *lon* and *cpxR* genes deleted veterinary vaccines against *Salmonella* in poultry and swine industries are an important step in preventing the spread of infection to humans through consumption of contaminated meat and poultry eggs [48] [51] [52].

References

[1] Dhanoa, A. and Fatt, Q.K. (2009) Non-Typhoidal *Salmonella* Bacteraemia: Epidemiology, Clinical Characteristics and Its' Association with Severe Immunosuppression. *Annals of Clinical Microbiology and Antimicrobials*, **8**, 15. http://dx.doi.org/10.1186/1476-0711-8-15

[2] Matheson, N., Kingsley, R.A., Sturgess, K., Aliyu, S.H., Wain, J., *et al*. (2010) Ten Years Experience of *Salmonella* Infections in Cambridge, UK. *Journal of Infection*, **60**, 21-25. http://dx.doi.org/10.1016/j.jinf.2009.09.016

[3] Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., *et al*. (2011) Foodborne Illness Acquired in the United States—Major Pathogens. *Emerging Infectious Diseases*, **17**, 7-15. http://dx.doi.org/10.3201/eid1701.P11101

[4] Hale, C.R., Scallan, E., Cronquist, A.B., Dunn, J., Smith, K., *et al*. (2012) Estimates of Enteric Illness Attributable to Contact with Animals and Their Environments in the United States. *Clinical Infectious Diseases*, **54**, S472-S479. http://dx.doi.org/10.1093/cid/cis051

[5] Takaya, A., Tomoyasu, T., Tokumitsu, A., Morioka, M. and Yamamoto, T. (2002) The ATP Dependent Lon Protease of *Salmonella enterica* Serovar Typhimurium Regulates Invasion and Expression of Genes Carried on *Salmonella* Pathogenicity Island 1. *Journal of Bacteriology*, **184**, 224-232. http://dx.doi.org/10.1128/JB.184.1.224-232.2002

[6] Darwin, K.H. and Miller, V.L. (1999) Molecular Basis of the Interaction of *Salmonella* with the Intestinal Mucosa.

Clinical Microbiology Reviews, **12**, 405-428.

[7] Hansen-Wester, I. and Hensel, M. (2001) *Salmonella* Pathogenicity Island Encoding Type III Secretion Systems. *Microbes and Infection*, **3**, 549-559. http://dx.doi.org/10.1016/S1286-4579(01)01411-3

[8] Beal, R.K., Powers, C., Davison, T.F., Barrow, P.A. and Smith, A.L. (2006) Clearance of Enteric *Salmonella enterica* Serovar Typhimurium in Chickens is Independent of B-Cell Function. *Infection and Immunity*, **74**, 1442-1444. http://dx.doi.org/10.1128/IAI.74.2.1442-1444.2006

[9] Curtiss, R., Kelly, S.M. and Hassan, J.O. (1993) Live Oral Avirulent *Salmonella* Vaccines. *Veterinary Microbiology*, **37**, 397-405. http://dx.doi.org/10.1016/0378-1135(93)90038-9

[10] Okamura, M., Tachizaki, H., Kubo, T., Kikuchi, S., Suzuki, A., *et al*. (2007) Comparative Evaluation of a Bivalent Killed *Salmonella vaccine* to Prevent Egg Contamination with *Salmonella enterica* Serovars Enteritidis, Typhimurium, and Gallinarum Biovar Pullorum, Using 4 Different Challenge Models. *Vaccine*, **25**, 4837-4844. http://dx.doi.org/10.1016/j.vaccine.2007.03.004

[11] Takaya, A., Suzuki, M., Matsui, H., Tomoyasu, T., Sashinami, H., *et al*. (2003) Lon, a Stress-Induced ATP-Dependent Protease, Is Critically Important for Systemic *Salmonella enterica* Serovar Typhimurium Infection of Mice. *Infection and Immunity*, **71**, 690-696. http://dx.doi.org/10.1128/IAI.71.2.690-696.2003

[12] Matsui, H., Suzuki, M., Isshiki, Y., Kodama, C., Eguchi, M., *et al*. (2003) Oral Immunization with ATP-Dependent Protease-Deficient Mutants Protects Mice against Subsequent Oral Challenge with Virulent *Salmonella enterica* Serovar Typhimurium. *Infection and Immunity*, **71**, 30-39. http://dx.doi.org/10.1128/IAI.71.1.30-39.2003

[13] Kim, S.W., Moon, K.H., Baik, H.S., Kang, H.Y., Kim, S.K., *et al*. (2009) Changes of Physiological and Biochemical Properties of *Salmonella enterica* Serovar Typhimurium by Deletion of *cpxR* and *lon* Genes Using Allelic Exchange Method. *Journal of Microbiological Methods*, **79**, 314-320. http://dx.doi.org/10.1016/j.mimet.2009.09.025

[14] Matsuda, K., Chaudhari, A.A., Kim, S.W., Lee, K.M. and Lee, J.H. (2010) Physiology, Pathogenicity and Immunogenicity of Lon and/or cpxR Deleted Mutants of *Salmonella gallinarum* as Vaccine Candidates for Fowl Typhoid. *Veterinary Research*, **41**, 59. http://dx.doi.org/10.1051/vetres/2010031

[15] Nandre, R.M., Matsuda, K., Chaudhari, A.A., Kim, B. and Lee, J.H. (2012) A Genetically Engineered Derivative of *Salmonella enteritidis* as a Novel Live Vaccine Candidate for Salmonellosis in Chickens. *Research in Veterinary Science*, **93**, 596-603. http://dx.doi.org/10.1016/j.rvsc.2011.11.005

[16] Rep, M., van Dij, J.M., Suda, K., Schatz, G., Grivell, L.A., *et al*. (1996) Promotion of Mitochondrial Membrane Complex Assembly by a Proteolytically Inactive Yeast Lon. *Science*, **274**, 103-106. http://dx.doi.org/10.1126/science.274.5284.103

[17] Chung, C.H. (1993) Proteases of *Escherichia coli*. *Science*, **262**, 372-374. http://dx.doi.org/10.1126/science.8211156

[18] Gottesman, S. (1996) Proteases and Their Targets in *Escherichia coli*. *Annual Review of Genetics*, **30**, 465-506. http://dx.doi.org/10.1146/annurev.genet.30.1.465

[19] Smith, C.K., Baker, T.A. and Sauger, R.T. (1999) Lon and Clp Family Protease and Chaperones Share Homologous Substrate-Recognition Domains. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 6678-6682. http://dx.doi.org/10.1073/pnas.96.12.6678

[20] Amerik, A.Y., Antonov, V.K., Gorbalenya, A.E., Kotova, S.A., Rotanova, T.V., *et al*. (1991) Site-Directed Mutagenesis of La Protease. A Catalytically Active Serine Residue. *FEBS Letters*, **287**, 211-214. http://dx.doi.org/10.1016/0014-5793(91)80053-6

[21] Goldberg, A.L. (1992) The Mechanism and Functions of ATP-Dependent Proteases in Bacterial and Animal Cells. *European Journal of Biochemistry*, **203**, 9-23. http://dx.doi.org/10.1111/j.1432-1033.1992.tb19822.x

[22] Takaya, A., Kubota, Y., Isogai, E. and Yamamoto, T. (2005) Degradation of the HilC and HilD Regulator Proteins by ATP-Dependent Lon Protease Leads to Downregulation of *Salmonella* Pathogenicity Island 1 Gene Expression. *Molecular Microbiology*, **55**, 839-852. http://dx.doi.org/10.1111/j.1365-2958.2004.04425.x

[23] Kultz, D. (2005) Molecular and Evolutionary Basis of the Cellular Stress Response. *Annual Review of Physiology*, **67**, 225-257. http://dx.doi.org/10.1146/annurev.physiol.67.040403.103635

[24] Majdalani, N. and Gottesman, S. (2005) The Rcs Phosphorelay: A Complex Signal Transduction System. *Annual Review of Microbiology*, **59**, 379-405. http://dx.doi.org/10.1146/annurev.micro.59.050405.101230

[25] Ngo, J.K. and Davies, K.J. (2009) Mitochondrial Lon Protease Is a Human Stress Protein. *Free Radical Biology and Medicine*, **46**, 1042-1048. http://dx.doi.org/10.1016/j.freeradbiomed.2008.12.024

[26] Langklotz, S. and Narberhaus, F. (2011) The *Escherichia coli* Replication Inhibitor CspD Is Subject to Growth-Regulated Degradation by the Lon Protease. *Molecular Microbiology*, **80**, 1313-1325. http://dx.doi.org/10.1111/j.1365-2958.2011.07646.x

[27] Flanagan, J.M. and Bewley, M.C. (2002) Protein Quality Control in Bacterial Cells: Integrated Networks of Chape-

rones and ATP-Dependent Proteases. *Genetic Engineering*, **24**, 17-47. http://dx.doi.org/10.1007/978-1-4615-0721-5_2

[28] Torres-Cabassa, A.S. and Gottesman, S. (1987) Capsule Synthesis in *Escherichia coli* K-12 Is Regulated by Proteolysis. *Journal of Bacteriology*, **169**, 981-989.

[29] Schmidt, R., Decatur, A., Rather, P., Moran Jr., C. and Losick, R. (1994) *Bacillus subtilis lon* Protease Prevents Inappropriate Transcription of Genes under the Control of the Sporulation Transcription Factor Sigma G. *Journal of Bacteriology*, **176**, 6528-6537.

[30] Wright, R., Stephens, C., Zweiger, G., Shapiro, L., Alley, A., *et al*. (1996) Caulobacter Lon Protease Has a Critical Role in Cell-Cycle Control of DNA Methylation. *Genes & Development*, **10**, 1532-1542. http://dx.doi.org/10.1101/gad.10.12.1532

[31] Stewart, B., Enos-Berlage, J. and McCarter, L. (1997) The *lonS* Gene Regulates Swarmer Cell Differentiation of *Vibrio parahaemolyticus*. *Journal of Bacteriology*, **179**, 107-114.

[32] Bretz, J., Losada, L., Lisboa, K. and Hutcheson, S. (2002) Lon Protease Functions as a Negative Regulator of Type III Protein Secretion in *Pseudomonas syringae*. *Molecular Microbiology*, **45**, 397-409.

[33] Kuroda, A., Nomura, K., Ohtomo, R., Kato, J., Ikeda, T., *et al.* (2001) Role of Inorganic Polyphosphate in Promoting Ribosomal Protein Degradation by the Lon Protease in *E. coli*. *Science*, **293**, 705-708.

[34] Christensen, S., Maenhaut-Michel, G., Mine, N., Gottesman, S., Gerdes, K., *et al*. (2004) Overproduction of the Lon Protease Triggers Inhibition of Translation in *Escherichia coli*: Involvement of the *yefM-yoeB* Toxin-Antitoxin System. *Molecular Microbiology*, **51**, 1705-1717.

[35] Silhavy, T.J., Kahne, D. and Walker, S. (2010) The Bacterial Cell Envelope. *Cold Spring Harbor Perspectives in Biology*, **2**, Article ID: a000414. http://dx.doi.org/10.1101/cshperspect.a000414

[36] Erickson, J.W. and Gross, C.A. (1989) Identification of the Sigma E Subunit of *Escherichia coli* RNA Polymerase: A Second Alternate Sigma Factor Involved in High-Temperature Gene Expression. *Genes & Development*, **3**, 1462-1471. http://dx.doi.org/10.1101/gad.3.9.1462

[37] Lipinska, B., Sharma, S. and Georgopoulos, C. (1988) Sequence Analysis and Regulation of the *htrA* Gene of *Escherichia coli*: A Sigma 32-Independent Mechanism of Heat-Inducible Transcription. *Nucleic Acids Research*, **16**, 10053-10067. http://dx.doi.org/10.1093/nar/16.21.10053

[38] Pogliano, J., Lynch, A.S., Belin, D., Lin, E.C. and Beckwith, J. (1997) Regulation of *Escherichia coli* Cell Envelope Proteins Involved in Protein Folding and Degradation by the Cpx Two-Component System. *Genes & Development*, **11**, 1169-1182. http://dx.doi.org/10.1101/gad.11.9.1169

[39] Raivio, T.L. and Silhavy, T.J. (1997) Transduction of Envelope Stress in *Escherichia coli* by the Cpx Two-Component System. *Journal of Bacteriology*, **179**, 7724-7733.

[40] Raffa, R.G. and Raivio, T.L. (2002) A Third Envelope Stress Signal Transduction Pathway in *Escherichia coli*. *Molecular Microbiology*, **45**, 1599-1611. http://dx.doi.org/10.1046/j.1365-2958.2002.03112.x

[41] MacRitchie, D.M., Buelow, D.R., Price, N.L. and Raivio, T.L. (2008) Two-Component Signaling and Gram Negative Envelope Stress Response Systems. *Advances in Experimental Medicine and Biology*, **631**, 80-110. http://dx.doi.org/10.1007/978-0-387-78885-2_6

[42] Stock, A.M., Robinson, V.L. and Goudreau, P.N. (2000) Two Component Signal Transduction. *Annual Review of Biochemistry*, **69**, 183-215. http://dx.doi.org/10.1146/annurev.biochem.69.1.183

[43] Dorel, C., Lejeune, P. and Rodrigue, A. (2006) The Cpx System of *Escherichia coli*, a Strategic Signaling Pathway for Confronting Adverse Conditions and for Settling Biofilm Communities? *Research in Microbiology*, **157**, 306-314. http://dx.doi.org/10.1016/j.resmic.2005.12.003

[44] Hung, D.L., Raivio, T.L., Jones, C.H., Silhavy, T.J. and Hultgren, S.J. (2001) Cpx Signaling Pathway Monitors Biogenesis and Affects Assembly and Expression of *P pili*. *The EMBO Journal*, **20**, 1508-1518. http://dx.doi.org/10.1093/emboj/20.7.1508

[45] Wolfe, A.J., Parikh, N., Lima, B.P. and Zemaitaitis, B. (2008) Signal Integration by the Two Competent Signal Transduction Response Regulator CpxR. *Journal of Bacteriology*, **190**, 2314-2322. http://dx.doi.org/10.1128/JB.01906-07

[46] Takaya, A., Suzuki, A., Kikuchi, Y., Eguchi, M., *et al*. (2005) Derepression of *Salmonella* Pathogenicity Island 1 Genes within Macrophages Leads to Rapid Apoptosis via Caspase-1- and Caspase-3-Dependent Pathways. *Cellular Microbiology*, **7**, 79-90. http://dx.doi.org/10.1111/j.1462-5822.2004.00435.x

[47] Nakayama, S., Kushiro, A., Asahara, T., Tanaka, R., Hu, L., *et al*. (2003) Activation of *hilA* Expression at Low pH Requires the Signal Sensor CpxA, but Not the Cognate Response Regulator CpxR, in *Salmonella enterica* Serovar Typhimurium. *Microbiology*, **149**, 2809-2817.

[48] Hur, J. and Lee, J.H. (2010) Immunization of Pregnant Sows with a Novel Virulence Gene Deleted Live *Salmonella*

Vaccine and Protection of Their Suckling Piglets against Salmonellosis. *Veterinary Microbiology*, **143**, 270-276.

[49] Jeon, B.W., Jawale, C.V., Kim, S.H. and Lee, J.H. (2012) Attenuated *Salmonella* Gallinarum Secreting an *Escherichia coli* Heat-Labile Enterotoxin B Subunit Protein as an Adjuvant for Oral Vaccination against Fowl Typhoid. *Veterinary Immunology and Immunopathology*, **150**, 149-160. http://dx.doi.org/10.1016/j.vetimm.2012.09.006

[50] Nandre, R.M., Jawale, C.V. and Lee, J.H. (2013) Enhanced Protective Immune Responses against *Salmonella* Enteritidis Infection by *Salmonella* Secreting an *Escherichia coli* Heat-Labile Enterotoxin B Subunit Protein. *Comparative Immunology, Microbiology and Infectious Diseases*, **36**, 536-548. http://dx.doi.org/10.1016/j.cimid.2013.06.002

[51] Nandre, R.M., Jawale, C.V. and Lee, J.H. (2013) Adjuvant Effect of *Escherichia coli* Heat Labile Enterotoxin B Subunit against Internal Egg Contamination in Domestic Fowl Immunized with a Live *Salmonella enteric* Serovar Enteritidis Vaccine. *The Veterinary Journal*, **197**, 861-867. http://dx.doi.org/10.1016/j.tvjl.2013.03.024

[52] Nandre, R., Matsuda, K. and Lee, J.H. (2014) Efficacy for a New Live Attenuated *Salmonella enteritidis* Vaccine Candidate to Reduce Internal Egg Contamination. *Zoonoses and Public Health*, **61**, 55-63. http://dx.doi.org/10.1111/zph.12042