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Original Research Article

Yersinia Enterocolatica as Vaccine Termed Bacterial Ghosts

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Abstract: *Yersinia enterocolitica* is a bacterial pathogenic organism which distributed widely in several countries including Iraq, among a broad range of environmental and animal reservoirs as cattle. In last decades, this bacterium has gained an increasing attention due to its role as an emerging foodborne pathogen within the context of globalized food supply chains and antimicrobial resistance. Hence, this stud aims to isolate *Y. enterocolitica* from the diarrheic cattle, preparation of bacterial ghosts (BGs) from this bacterium and experimental evaluation its efficiency in stimulating immune response in mice. Initially, fresh feces were obtained from 87 diarrheic cattle at Al-Diwaniyah city (Al-Qadisiya, Iraq) during November (2023)-May (2024), and processed to isolate *Y. enterocolitica* strain that used to preparation stocks of *Y. enterocolitica* ghosts (YEGs). Then, a total of 30 mice were selected and divided into NCG (not injected YEGs), SCG (mice injected an adjuvant YEGs (70µg/100µl), subcutaneously), and IMG (mice injected an adjuvant YEGs (70µg/100µl), intramuscularly). The mice were injected once at 0, 2, 4 and 8 weeks of study experiments. Then, blood samples were drained from all study mice to pipette sera for serologicy using ELISA to measurement the levels of serum IL-1α, IL-2, and IFN-γ. Our findings revealed that 16.09% of study cattle were positively infected by In *Y. enterocolitica*. Experimentally, the findings of IL-1α were shown a significant reduction in values of SCG (11.99±2.38pg/ml) but insignificant variation ($p>0.05$) in values of IMG (59.74±12.2pg/ml) in comparison to values of NCG (61.68±12.38pg/ml). Concerning IL-2, values of experimental groups, SCG (8.98±1.24pg/mL) and IMG (8.11±1.44pg/mL) were obviously greater than NCG (2.92±0.32pg/mL). Significant elevation of IFN-γ was recorded in experimental groups, SCG (282.61±28.3pg/mL) and IMG (370.24±20.5pg/mL), when compared to values of NCG (104.30±25.4pg/mL). Among experimentally groups, values of IMG were much more than SCG.This study indicated the robust adjuvant properties of YEGs as a vaccine in inducing of immune response and enhancing of immunogenicity, making them potent stimulators of both innate and adaptive immune responses.

Keywords: Diarrheic Cattle, Immune Response, Sponge-Like Reduced Protocol, Enzyme-Linked Immunosorbent Assay (ELISA), Iraq.

INTRODUCTION

Yersinia enterocolitica is a Gram-negative, facultatively anaerobic bacterium belonging to the family Enterobacteriaceae, recognized as a significant zoonotic and foodborne pathogen of global public health concern with causing a disease known as yersiniosis (Azam *et al*., 2023). This bacterium represents one of the three major pathogenic species within the genus *Yersinia*, alongside *Y. pestis* and *Y. pseudotuberculosis*, although only certain strains of *Y. enterocolitica* are pathogenic to humans (Robins-Browne, 2012; Saraiva *et al*., 2025). The disease caused by *Y. enterocolitica* primarily manifests as gastrointestinal illness, including diarrhea, terminal ileitis, and mesenteric lymphadenitis (Fàbrega Santamaria, 2010). The pathogenicity of *Y. enterocolitica* is mediated through a range of virulence factors that facilitate colonization, invasion, and survival within the host. Following ingestion of contaminated food or water, the bacterium colonizes the intestinal mucosa, particularly the ileum and invades Peyer's patches and mesenteric lymph nodes. Key virulence determinants include the virulence plasmid that encodes *Yersinia* outer proteins (Yops), as well as chromosomal genes such as *ail* (attachment and invasion locus), *inv* (invasion), and *yodA* (adhesion), all of which contribute to immune evasion and intracellular survival (Drummond *et al*., 2012; Ahmed *et al*., 2019).

Despite its clinical and public health significance, there is currently no widely licensed vaccine available for the prevention of yersiniosis. Consequently, preventive strategies rely primarily on food safety measures, hygiene practices, and control of infection in animal reservoirs (Andey *et al*., 2024). Nevertheless, considerable research has focused on the development of effective vaccines targeting *Y. enterocolitica* particularly in the context of its virulence factors and immunogenic antigens (Yadav *et al*., 2020; Solana *et al*., 2022). One of the most promising and innovative strategies in vaccine development against *Y. enterocolitica* involves the use of bacterial ghost (BG) technology. BGs are empty, non-living bacterial cell envelopes produced through the controlled expression of the lysis gene *E* derived from the bacteriophage ΦX174 (Hajam *et al*., 2017; Chen *et al*., 2021; Anwer *et al*., 2025). This process results in the formation of transmembrane tunnels that expel cytoplasmic contents while preserving the structural integrity of the bacterial cell wall including surface antigens and immunogenic components. As a result, BGs retain the native antigenic profile of the pathogen without the risk of replication or infection (Faghihkhorasani *et al*., 2023; Park, 2023). The use of BGs as vaccine candidates offers several advantages. First, the present multiple native antigens in their structural conformation, enhancing immunogenicity compared to purified subunit vaccines. Second, they can stimulate both innate and adaptive immune responses such as mucosal immunity that particularly important for enteric pathogens. Third, BGs can serve as delivery systems for heterologous antigens or DNA, further expanding their activities in vaccine design (Hajam *et al*., 2017; Chi *et al*., 2024). Therefore, this study conducts to isolation *Y. enterocolitica* from diarrheic cattle, preparation of BGs from study isolates, and experimental evaluation its efficiency in stimulating immune response in mice.

MATERIALS AND METHODS

Ethical Approval

This study was licensed by and conducted under the supervision of the Scientific Committee in the Department of Microbiology (College of Veterinary Medicine, University of Al-Qadisiyah).

Samples

A total of 87 diarrheic cattle at various locations in Al-Diwaniyah city (Al-Qadisiyah, Iraq) were subjected to the current study during November (2023)-May (2024). Under aseptic conditions, fecal samples were collected from all study animals into disposable plastic containers, and transported as soon as possible to the Microbiology Lab in the Department of Microbiology (College of Veterinary Medicine).

Culture

In Microbiology Lab, the fecal samples were inoculated in 5 ml Nutrient broth (NB), incubated at 37ºC for 24 hours. Subsequently, the colonies were transported to selective agar to be incubated at 37ºC for 24-48 hours, MacConkey agar and blood agar, and then incubated overnight at 37ºC. The suspected isolates were further examined by the Gram stain under light microscope, and through the standard biochemical tests including catalase, urease, methyl red, motility, arabinose fermentation, Voges-Proskauer reaction, oxidase, indole production, Esculin hydrolysis, and citrate utilization (Quinn *et al*., 2011).

Preparation of Bacterial Ghost

Study isolates of *Y. enterocolitica* were re-cultivated in 500mL NB, incubated (37°C/72 hours) under static condition, and the cell biomass was collected by centrifugation. Then the cell biomass was washed (0.5% saline), and re-suspended by distilled water. To preparation of YEGs, the Sponge-Like Reduced Protocol was followed (Amara *et al*., 2013; Amara, 2023). Then, DNA and protein concentrations were determined in YEGs with further evaluation of YEGs by crystal violet staining and examination under light microscope. Finally, the prepared YEGs were evaluated for existence of any still viable cells by re-suspending on the Yersinia selective agar plates and incubation at 37°C for 48 hours.

Study Design

A total of 30 adult albino mice, 4 months age and 25-40grams weight, were purchased, transported to the Lab Animal House, acclimated for 7 days, and divided randomly as following;

1. Negative control group (NCG): This group involved 8 mice that not injected YEGs.
2. Experimental group 1 (SCG): This group composed 8 mice that injected subcutaneously a mixture of 70µg of YEGs/100µl mixed with 100µl complete Freund's adjuvant, once daily at 0, 2, 4 and 8 weeks of study experiment.
3. Experimental group 2 (IMG): This group composed 8 mice that injected intramuscularly a mixture of 70µg of YEGs/100µl mixed with 100µl complete Freund's adjuvant, once daily at 0, 2, 4 and 8 weeks of study experiment.

After ending of the experiment period, all study mice were anesthetized to collection of blood directly from heart using free-anticoagulant glass-gel tubes. Then, the blood tubes were centrifuged, and the obtained sera were pipetted and kept frozen at -20ºC until tested serologically by ELISA.

Immune Examination

Following the manufacturer instructions for IL-1α (E-EL-M3059), IL-2 (E-EL-M0042), and IFN-γ (E-EL-M0048) kits, the obtained sera of study mice and kit contents were prepared and processed step-by-step. The obtained values of optical density (ODs) for Standard solution diluents and sera were measured to calculate the concentrations of each marker through the Standard Curve.

Statistical Analysis

One-Way Analysis of Variance (ANOVA) in the GraphPad Prism Software was used to detected significant differences between the obtained values of experimental study groups at p<0.05 (*), p<0.01 (**), p<0.001 (***), and p<0.0001 (****). Values of study results were represented as Mean ± Standard Error (M±SE), (Al-Gharban and Al-Taee, 2016).

RESULTS

In the present study, *Y. enterocolitica* was isolated from the fecal samples of 16.09% (14/87) study cattle; while, 83.91% (73/87) were negatives (Figures 1, 2). Biochemically, the isolates of *Y. enterocolitica* were shown negative reactivity to Gram stain, motility test at 37°C, Esculin hydrolysis, oxidase, indole, and citrate; while positive activity was seen for catalase, methyl red, motility test at 25°C, urease, arabinose fermentation, and Voges-Proskauer.

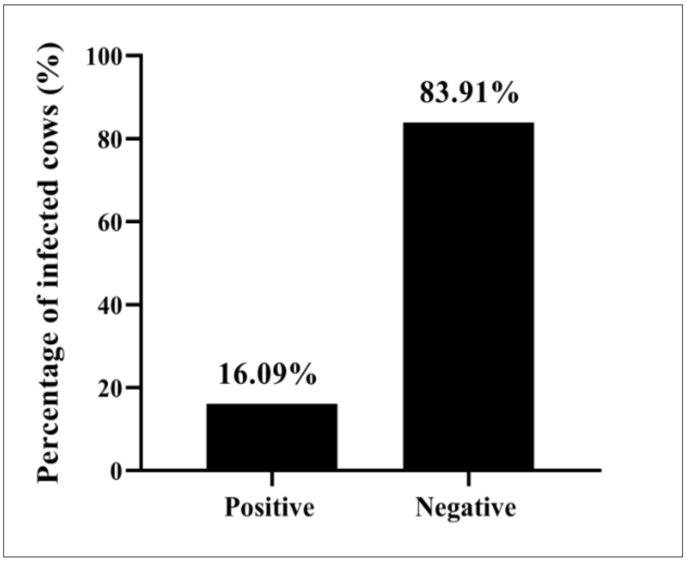

Figure 1: Prevalence rate of *Y. enterocolitica* in feces of overall 87 diarrheic cattle

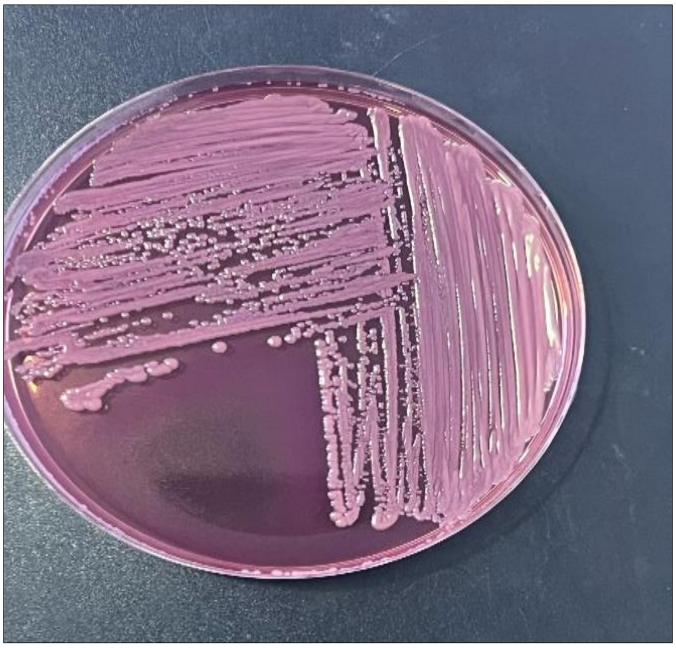

Figure 2: Colonies of *Y. enterocolitica* on selective media

Immune Markers in Mice

In comparison to values of NCG (61.68 ± 12.38pg/mL), the findings of IL-1α were shown a marked decrease in values of SCG (11.99 ± 2.38pg/mL) but insignificant variation (p>0.05) in values of IMG (59.74 ± 12.2pg/mL), (Table 1, Figure 1).

Table 1: Levels of IL-1α in mice of different study groups

Group	Value
NCG	61.68 ± 12.38
SCG	11.99 ± 2.38
IMG	59.74 ± 12.2 *
p-value	0.0116
LSD	31.23
95%CI	25.45 to 114.4

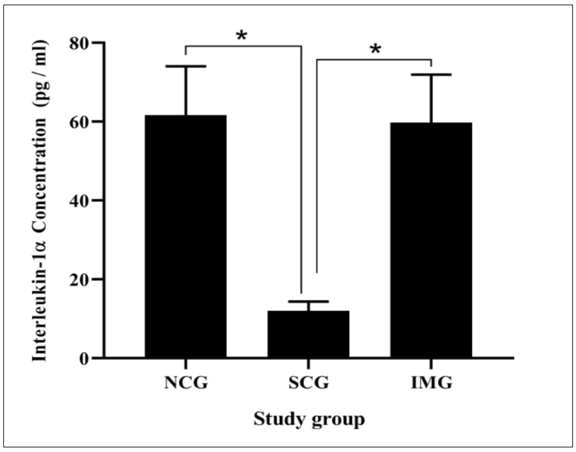

Figure 1: Concentration of serum IL-1α in mice of different study groups; NCG (negative control), SCG (experimental group one injected YEGs subcutaneously), and IMG (experimental group two injected YEGs intramuscularly); Significance (*)

Concerning IL-2, though insignificance ($p>0.05$) was identified among experimental groups, SCG (8.98 ± 1.24pg/mL) and IMG (8.11 ± 1.44pg/mL), the findings of both experimental groups were significantly ($p<0.05$) higher than those of NCG (2.92 ± 0.32pg/mL), (Table 2, Figure 2).

Table 2: Levels of IL-2 in mice of different study groups

Group	Value
NCG	2.92 ± 0.32
SCG	8.98 ± 1.24 *
IMG	8.11 ± 1.44 *
p-value	0.0319
LSD	3.26
95%CI	1.470 to 14.81

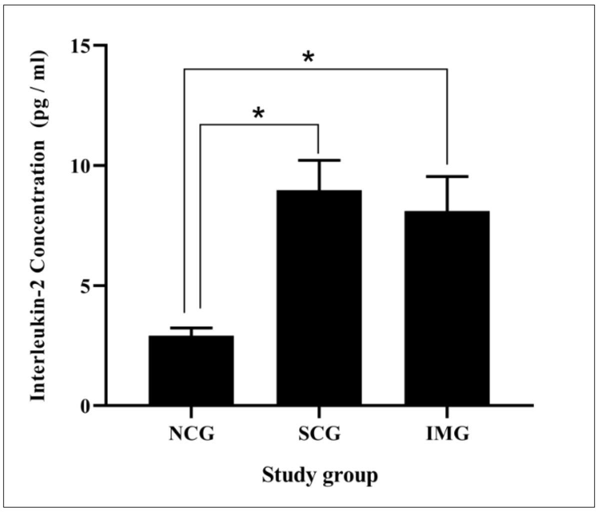

Figure 2: Concentration of serum IL-2 in mice of different study groups; NCG (negative control), SCG (experimental group one injected YEGs subcutaneously), and IMG (experimental group two injected YEGs intramuscularly); Significance (*)

Significantly, the findings of IFN-γ were revealed a marked elevation in both experimental group, SCG (282.61 ± 28.3pg/mL) and IMG (370.24 ± 20.5pg/mL), when compared to values of NCG (104.30 ± 25.4pg/mL). Among experimentally groups, values of IMG were apparently higher than SCG (Table 3, Figure 3).

Table 3: Levels of IFN-γ in mice of different study groups

Group	Value
NCG	104.30 ± 25.4
SCG	282.61 ± 28.3
IMG	370.24 ± 20.5 *
p-value	0.0242
LSD	72.9
95%CI	84.27 to 589.0

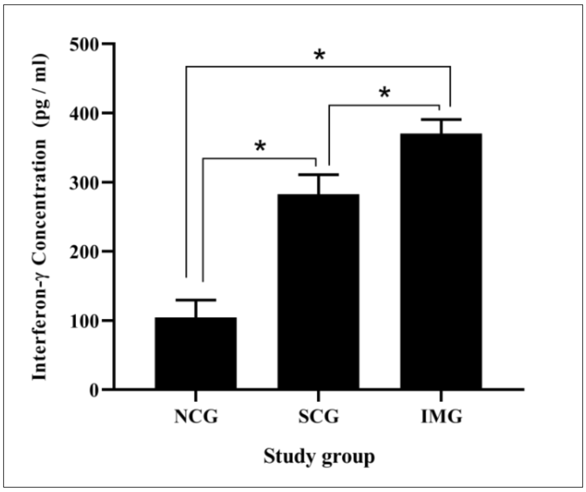

Figure 2: Concentration of serum IL-2 in mice of different study groups; NCG (negative control), SCG (experimental group one injected YEGs subcutaneously), and IMG (experimental group two injected YEGs intramuscularly); Significance (*)

DISCUSSION

Among 74 cattle, 16.09% of study cattle were shown a positive infectivity for *Y. enterocolitica*. Comparatively, several researchers have indicated implication of *Y. enterocolitica* in incidence of diarrhea (Zheng *et al*., 2008; Wang *et al*., 2019; Riahi *et al*., 2021; Gharban, 2024) and the role of livestock and wildlife animals as a source of infection (Shayegani *et al*., 1981; Turkson *et al*., 1988; Hurvell, 2018; Sotohy *et al*., 2024). In cattle, the numbers of prevalence studies are relatively low and *Y. enterocolitica* was mostly from milk or milk products (Khalid and Abbas, 2021; Yazdi *et al*., 2022; Dowidar and El-Baz, 2025), raw meat (Łada *et al*., 2023; Schmid e*t al*., 2025), and feces (O'Grady *et al.,* 2016; Bièche-Terrier *et al*., 2024).

Recent advances in molecular biology and genetic engineering have further improved the efficiency and safety of BG production. Experimentally, our findings revealed that injection of *Y. enterocolitica as BG* was contributed effectively in inducing of immune response. As inflammation is the basic defense response to various microbial infections, we suggest that YEGs have the potential to act as regulators of the innate and adaptive immunity that can defense from bacterial or viral infections. This is an agreement with the results reported by various studies that referred to BGs as a safe immunogenic vaccine (Fischer *et al*., 2021; Banks *et al*., 2023; Chen *et al*., 2023). Moreover, other researchers have demonstrated that BG contains higher activity to stimulating innate immunity, and acting as efficient adjuvant (Mayr *et al*., 2005; Jenab *et al*., 2020; Cognigni *et al*., 2021).

Cai *et al*., (2013) confirmed experimentally the marked elevation of IL-4/IFN-γ cytokines and IgG1/IgG2a antibodies in mice after administration of YEGs as a vaccine. Other studies have been demonstrated that upon exposure to YEGs, antigen presenting cells (APCs) such as macrophages and dendritic cells become activated and secrete a range of cytokines that are essential for initiating and shaping immune response. These include key pro-inflammatory cytokines such as TNF-α, il-1β, and IL-6 which contribute to the early inflammatory response and recruitment of immune cells to the site of antigen exposure (Drechsler-Hake *et al*., 2016; Bancerz-Kisiel *et al*., 2018; Silva *et al*., 2020; Gharban *et al*., 2023). Sasaki *et al*., (2022) recorded that BGs can promote the activation of CD4+ helper cells and CD8+ cytotoxic T lymphocytes, leading to increased secretion IL-2 and establishment of a robust cellular immune response.

Other researchers demonstrated the capability of BGs to serve as delivery platforms for heterologous antigens, DNA, or adjuvants for further enhancing their immune-stimulatory potential, and their ability to inducing pro-inflammatory cytokines to elimination or reducing the need for additional adjuvant compounds (Faghihkhorasani *et al*., 2023; Gan *et al*., 2023). Narayanan *et al*., (2024) demonstrated ability of BGs in modulation function of *Caenorhabditis elegans* and NK-92 cells, as well as perform as cytokine-therapy adjuvants. Subsequently, they reported that BGs significantly influenced the innate immune response through the upregulation of lysozyme genes *viz*., *ilys*-3 (8.8-fold) and *lys*-2 (3.1-fold). Resta *et al*., (2024) concluded that the preserved lipopolysaccharides and outer membrane components in YEGs can strongly activate pattern recognition receptors such as toll-like receptor 4 (TLR4) leading to the activation of nuclear factor kappa B signaling pathway and subsequent up-regulation of IL-1α expression. In a recent study, Filik-Matyjaszczyk *et al*., (2025) showed that YEGs can induce the production of interferons, particularly IFN-α/β and INF-γ which are critical for antiviral and antibacterial immunity by enhancing the macrophage activation and promotion to clearance of intracellular pathogens.

CONCLUSION

This study indicated the robust adjuvant properties of YEGs as a vaccine in inducing of immune response and enhancing of immunogenicity, making them potent stimulators of both innate and adaptive immune responses. However, furthermore studies are necessary to estimate the ability of YEGs to deliver vaccine antigens or therapeutic molecules directly to immune cells.

REFERENCES

- Ahmed, H. A., Tahoun, A. B., Abou Elez, R. M., Abd El-Hamid, M. I., and Abd Ellatif, S. S. (2019). Prevalence of Yersinia enterocolitica in milk and dairy products and the effects of storage temperatures on survival and virulence gene expression. *International dairy journal*, *94*, 16-21.
- Al-Gharban, H. A., and Al-Taee, H. S. (2016). Seroclinical diagnosis of Anaplasma marginale bacteria in carrier arabian one-humped camels. *Basrah J Vet Res*, *15*, 346-359.
- Amara, A. A., Salem-Bekhit, M. M., and Alanazi, F. K. (2013). Sponge-like: a new protocol for preparing bacterial ghosts. *The Scientific World Journal*, *2013*(1), 545741.
- Amara, A.A.A.F. (2023). Bacterial and Microbial Ghosts Preparation. *Biomedical Journal of Scientific and Technical Research*, *48*(2), 39607-39616.
- Andey, T., Soni, S., and Modi, S. (2024). Conventional vaccination methods: Inactivated and live attenuated vaccines. *Advanced Vaccination Technologies for Infectious and Chronic Diseases*, 37-50.
- Anwer, M., Bhaliya, K., Munn, A., and Wei, M. Q. (2025). Bacterial ghosts: A breakthrough approach to cancer vaccination. *Biomedicine and Pharmacotherapy*, *182*, 117766.
- Azam, M. W., Zarrilli, R., and Khan, A. U. (2023). Updates on the virulence factors produced by multidrug-resistant Enterobacterales and strategies to control their infections. *Microorganisms*, *11*(8), 1901.
- Bancerz-Kisiel, A., Pieczywek, M., Łada, P., and Szweda, W. (2018). The most important virulence markers of Yersinia enterocolitica and their role during infection. *Genes*, *9*(5), 235.
- Banks, C., Lindbom, B. J., Kitson, G., Darsley, M., and Fischer, P. B. (2023). Preclinical development of a novel Group B Streptococcus (GBS) vaccine candidate for maternal immunization based upon the alpha-like protein family of GBS surface proteins (Alp). *Birth Defects Research*, *115*(9), 933-944.
- Bièche-Terrier, C., Coadou, T., Lamour, B., Feurer, C., Le Guern, A. S., and Denis, M. (2024, December). Prévalence de Yersinia enterocolitica dans les fèces de bovins prélevés à l'abattoir. In *3R2024: 27èmes Rencontres autour des Recherches sur les Ruminants*.
- Cai, K., Zhang, Y., Yang, B., and Chen, S. (2013). Yersinia enterocolitica ghost with msbB mutation provides protection and reduces proinflammatory cytokines in mice. *Vaccine*, *31*(2), 334-340.
- Chen, H., Ji, H., Kong, X., Lei, P., Yang, Q., Wu, W., and Sun, D. (2021). Bacterial ghosts-based vaccine and drug delivery systems. *Pharmaceutics*, *13*(11), 1892.
- Chen, T., Ding, Z., Lan, J., and Wong, G. (2023). Advances and perspectives in the development of vaccines against highly pathogenic bunyaviruses. *Frontiers in cellular and infection microbiology*, *13*, 1174030.
- Chi, Y., Zhang, S., and Ji, S. (2024). Delivery of a hepatitis C virus vaccine encoding NS3 linked to the MHC class II chaperone protein invariant chain using bacterial ghosts. *Biomedicines*, *12*(3), 525.
- Cognigni, V., Ranallo, N., Tronconi, F., Morgese, F., and Berardi, R. (2021). Potential benefit of β-glucans as adjuvant therapy in immuno-oncology: A review. *Exploration of targeted anti-tumor therapy*, *2*(2), 122.
- Dowidar, H. A., and El-Baz, A. H. (2025). Genotype and Biofilm Patterns of Yersinia enterocolitica Isolated from Milk and Some Milk Products in Mansoura City, Dakahlia, Egypt. *Egyptian Journal of Veterinary Sciences*, *56*(13), 155-164.
- Drechsler-Hake, D., Alamir, H., Hahn, J., Günter, M., Wagner, S., Schütz, M., and Autenrieth, S. E. (2016). Mononuclear phagocytes contribute to intestinal invasion and dissemination of Yersinia

enterocolitica. *International Journal of Medical Microbiology*, *306*(6), 357-366.

- Drummond, N., Murphy, B. P., Ringwood, T., Prentice, M. B., Buckley, J. F., and Fanning, S. (2012). Yersinia enterocolitica: a brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. *Foodborne pathogens and disease*, *9*(3), 179-189.
- Fàbrega Santamaria, A. (2010). Mechanisms of fluoroquinolone resistance in Escherichia coli, Salmonella Typhimurium and Yersinia enterocolitica. Influence on expression of virulence factors. PhD Thesis, Departament Anatomia Patològica, Farmacologia i Microbiologia, Universitat de Barcelona, Spain.
- Faghihkhorasani, A., Ahmed, H. H., Mashool, N. M., Alwan, M., Assefi, M., Adab, A. H., and Baghani, M. (2023). The potential use of bacteria and bacterial derivatives as drug delivery systems for viral infection. *Virology Journal*, *20*(1), 222.
- Faghihkhorasani, A., Ahmed, H. H., Mashool, N. M., Alwan, M., Assefi, M., Adab, A. H., and Baghani, M. (2023). The potential use of bacteria and bacterial derivatives as drug delivery systems for viral infection. *Virology Journal*, *20*(1), 222.
- Filik-Matyjaszczyk, K., Matyjaszczyk, I., Ciesielska, M., Szermer-Olearnik, B., Mikołajczyk, K., and Gamian, A. (2025). Phage receptor binding protein and Fc fragment fusion enhances phagocytosis of Y. enterocolitica. *AMB Express*, *15*(1), 135.
- Fischer, P., Pawlowski, A., Cao, D., Bell, D., Kitson, G., Darsley, M., and Johansson-Lindbom, B. (2021). Safety and immunogenicity of a prototype recombinant alpha-like protein subunit vaccine (GBS-NN) against Group B Streptococcus in a randomised placebo-controlled double-blind phase 1 trial in healthy adult women. *Vaccine*, *39*(32), 4489-4499.
- Gan, Y., Zhao, G., Wang, Z., Zhang, X., Wu, M. X., and Lu, M. (2023). Bacterial membrane vesicles: physiological roles, infection immunology, and applications. *Advanced Science*, *10*(25), 2301357.
- Gharban, H. A. (2024). First genotyping confirmation of Pichia kudriavzevii in subclinically mastitic cows in Iraq. *Revista de Ciências Agroveterinárias*, *23*(3), 417-424.
- Gharban, H. A., Al-Ghuraibawi, H. N., Al-Rubaye, Z. A., Jahlol, H. A., Al-Zergany, A. A., and Al-Abedi, G. J. (2023). Most clinically detected viral diseases in field animals of Wasit Province, Iraq. *Annals of the Romanian Society for Cell Biology*, *27*(01), 154-168.
- Hajam, I. A., Dar, P. A., Won, G., and Lee, J. H. (2017). Bacterial ghosts as adjuvants: mechanisms and potential. *Veterinary Research*, *48*(1), 37.
- Hurvell, B. (2018). Zoonotic Yersinia enterocolitica infection: host range, clinical manifestations, and transmission between animals and man. In *Yersinia enterocolitica* (pp. 145-159). CRC Press.
- Jalava, K., Hensel, A., Szostak, M., Resch, S., and Lubitz, W. (2002). Bacterial ghosts as vaccine candidates for veterinary applications. *Journal of Controlled Release*, *85*(1-3), 17-25.
- Jenab, A., Roghanian, R., and Emtiazi, G. (2020). Bacterial natural compounds with anti-inflammatory and immunomodulatory properties (mini review). *Drug design, development and therapy*, 3787-3801.
- Khalid, D. M., and Abbas, B. A. (2021). Prevalence, antibiotic susceptibility, and virulence factors of Yersinia enterocolitica isolated from raw milk in Basrah, Iraq. *Bulgarian Journal of Veterinary Medicine*, *24*(1).
- Łada, P., Kończyk-Kmiecik, K., and Bancerz-Kisiel, A. (2023). Isolation, characterization and antimicrobial resistance of Yersinia enterocolitica from Polish cattle and their carcasses. *BMC veterinary research*, *19*(1), 143.
- Mayr, U. B., Walcher, P., Azimpour, C., Riedmann, E., Haller, C., and Lubitz, W. (2005). Bacterial ghosts as antigen delivery vehicles. *Advanced drug delivery reviews*, *57*(9), 1381-1391.
- Narayanan, S., Baburajan, A. P., Muhammad, M., Joseph, A., Vemula, P. K., and Bhat, S. G. (2024). Demonstrating the immunostimulatory and cytokine-augmentation effects of bacterial ghosts on natural killer cells and Caenorhabditis Elegans. *Biotechnology and bioengineering*, *121*(3), 959-970.
- O'Grady, D., Kenny, K., Power, S., Egan, J., and Ryan, F. (2016). Detection of Yersinia enterocolitica serotype O: 9 in the faeces of cattle with false positive reactions in serological tests for brucellosis in Ireland. *The Veterinary Journal*, *216*, 133-135.
- Park, S. Y. (2023). Chemically induced bacterial ghosts: a novel approach for advancing biomedical applications. *Molecular and Cellular Toxicology*, *19*(4), 657-665.
- Resta, S. C., Guerra, F., Talà, A., Bucci, C., and Alifano, P. (2024). Beyond inflammation: Role of pyroptosis pathway activation by gram-negative bacteria and their outer membrane vesicles (OMVs) in the interaction with the host cell. *Cells*, *13*(21), 1758.
- Riahi, S. M., Ahmadi, E., and Zeinali, T. (2021). Global prevalence of Yersinia enterocolitica in cases of gastroenteritis: A systematic review and meta-analysis. *International journal of microbiology*, *2021*(1), 1499869.
- Robins-Browne, R. M. (2012). Yersinia enterocolitica. *Food microbiology: Fundamentals and frontiers*, 339-376.
- Saraiva, S., García-Díez, J., de Sousa, T., Calouro, R., Fernandes, D., Mourão, A. V., and Poeta, P. (2025). Zoonotic Yersinia enterocolitica in Swine: One Health Implications for Food Safety and Public Health. *Veterinary Sciences*.

- Sasaki, K., Moussawy, M. A., Abou-Daya, K. I., Macedo, C., Hosni-Ahmed, A., Liu, S., and Abdelsamed, H. A. (2022). Activated-memory T cells influence naïve T cell fate: a noncytotoxic function of human CD8 T cells. *Communications Biology*, *5*(1), 634.
- Schmid, D. T., Barmettler, K., Stevens, M. J., and Stephan, R. (2025, October). Occurrence and Characteristics of Salmonella spp., L. monocytogenes, Y. enterocolitica, and Campylobacter spp. in Raw Meat-Based Diets for Pets. In *Pets* (Vol. 2, No. 4, p. 35). MDPI.
- Shayegani, M. E. H. D. I., DeForge, I. S. O. B. E. L., McGLYNN, D. M., and Root, T. (1981). Characteristics of Yersinia enterocolitica and related species isolated from human, animal, and environmental sources. *Journal of Clinical microbiology*, *14*(3), 304-312.
- Silva, J. E., Mayordomo, A. C., Dave, M. N., Aguilera Merlo, C., Eliçabe, R. J., and Di Genaro, M. S. (2020). Dendritic cells of mesenteric and regional lymph nodes contribute to Yersinia enterocolitica O: 3–induced reactive arthritis in TNFRp55−/− mice. *The Journal of Immunology*, *204*(7), 1859-1868.
- Solana, J. C., Moreno, J., Iborra, S., Soto, M., and Requena, J. M. (2022). Live attenuated vaccines, a favorable strategy to provide long-term immunity against protozoan diseases. *Trends in Parasitology*, *38*(4), 316-334.
- Sotohy, S. A., Diab, M. S., Ewida, R. M., Aballah, A., and Eldin, N. K. A. (2024). An investigative study on Yersinia enterocolitica in animals, humans and dried milk in New Valley Governorate, Egypt. *BMC microbiology*, *24*(1), 395.
- Turkson, P. K., Lindqvist, K. J., and Kapperud, G. (1988). Isolation of Campylobacter spp. and Yersinia enterocolitica from domestic animals and human patients in Kenya. *Apmis*, *96*(1-6), 141-146.
- Wang, E. W., Bhatti, M., Cantu, S., and Okhuysen, P. C. (2019, April). Diagnosis of Yersinia enterocolitica infection in cancer patients with diarrhea in the era of molecular diagnostics for gastrointestinal infections. In *Open forum infectious diseases* (Vol. 6, No. 4, p. ofz116). US: Oxford University Press.
- Yadav, D. K., Yadav, N., and Khurana, S. M. P. (2020). Vaccines: present status and applications. In *Animal biotechnology* (pp. 523-542). Academic Press.
- Yazdi, S. S., Mobasseri, B., Bonab, P. T., Yazdi, S. S., Mirbagheri, Z., and Dallal, M. M. S. (2022). Prevalence and characteristics of Yersinia enterocolitica and Yersinia pseudotuberculosis from raw milk supplied in Tehran. *Journal of nutrition and food security*.
- Zheng, H., Sun, Y., Lin, S., Mao, Z., and Jiang, B. (2008). Yersinia enterocolitica infection in diarrheal patients. *European Journal of Clinical Microbiology and Infectious Diseases*, *27*(8), 741-752.