Review

*Helicobacter pullorum*: A potential hurdle emerging pathogen for public health

Wafaa A Abd El-Ghany¹

¹Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract

Emerging zoonotic pathogens gain more attention due to the adverse effects on human and animal’s health and productivity. One of these zoonotic pathogens is *Helicobacter pullorum* (*H. pullorum*) which was firstly diagnosed in 1994. This bacterium is enteropathogenic in poultry and contaminates the carcasses meat during processing or improper handling. Human can get *H. pullorum* infection mainly through mishandling of contaminated carcasses or consumption of undercooked meat. Infection of *H. pullorum* in human is associated with gastroenteritis and hepatitis. Diagnosis of *H. pullorum* is very difficult as misdiagnosis with other enteric zoonotic pathogens like *Campylobacter* and other *Helicobacter* species is common. Unlike other types of *Helicobacter*, there are little information and few researches regarding prevalence, pathogenesis, diagnosis and control of *H. pullorum* infection either animals or human. Accordingly, this review article was prepared to give more details about *H. pullorum* sources of infection, pathogenicity, incidence in poultry and human as well as its treatment.

Key words: *H. pullorum*; human; poultry; public health; zoonosis.

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Introduction

Emerging disease is that caused by new etiological agent previously known but now occurring in species or places where the disease was unknown [1]. Infection with *Helicobacter* species is considered as one of these emerging diseases. Genus *Helicobacter* is belonging to class *Epsilonproteobacteria* that was established in 1989. These pathogens are divided into two groups, gastric and enterohpatic, based on their preferred site of colonization [2] and also on 16S rRNA sequence data. More than 30 species of *Helicobacter* have been recorded in the last two decades [3]. One of these newly identified species is *Helicobacter pullorum* (*H. pullorum*). This pathogen is fastidious, microaerophilic, non-sporulated and Gram-negative spirally curved motile bacillus with monopolar flagellae [2]. It has been considered that *H. pullorum* is an enterohpatic *Helicobacter* species [4]. This bacterium has been early discovered from the intestine and liver of diarrheic birds [5,6] as well as from the faeces and biopsies of patients with gastroenteritis, chronic liver disease and inflammatory bowel disease [7]. *H. pullorum* has a zoonotic potential [8-12] as it has been associated with approximately 12% of human zoonotic cases [13]. Consumption of undercooked or surface contaminated chicken is considered as a potential route of *Helicobacter* transmission to human beings [14]. Susceptibility of avian and animal species as well as human beings to *H. pullorum* is variable. Infections with *H. pullorum* were recorded in different avian species like chickens, turkeys, ostriches, Guinea fowl, parrots and psittacine birds [15,16], rabbits and rodents [17-19] and human [20]. Limited sources of data concerning *H. pullorum* infection in different species and their relations to human infection are available. Thus, in this review article, we will investigate *H. pullorum* sources of infection, pathogenicity, incidence in poultry and human as well as the possible treatment of this pathogen.

Sources of infection

The sources of *H. pullorum* infection to human is summarized in Table 1. Avian species serve as potent reservoirs for *H. pullorum* [21]. There is an association between *H. pullorum* presence in the intestinal tract of poultry with diarrhea and vibricion hepatitis as well as presence of pathogen in patients with diarrhea, vomiting and liver and gallbladder diseases [2,7,22]. Surface contamination of broiler chickens’ carcasses with the caecal contents during processing and handling is common [2,5]. It has been detected that *H. pullorum* colonizes the caecum of broilers and is excreted in the
droppings till slaughtering and this implies that chicken meat constitutes a major source of infection for human [23]. Therefore, *H. pullorum* is considered as a pathogen of food borne significance [24,25]. In Australia, *H. pullorum* was isolated from chicken meat in the rate of 13.5% [26]. Furthermore, González et al. [27] identified *H. pullorum* in 3 types of chicken’s meat products with 99% genetic match. Similarly, Borges et al. [10] in Italy demonstrated that the emerging *H. pullorum* pathogen can be transmitted to humans by chicken meat consumption and/or contact as the organism was isolated from 4 out of 17 (23.5%) fresh chicken meat samples from different producers.

It should be noted that, not only poultry meat is the source of *H. pullorum* infection for human, but also table eggs are another source. A total of 300 commercial chicken eggs were collected from Assiut and Qena governorates in Egypt for the presence of *Helicobacter* species detection [4]. The authors found that *H. pullorum* contamination rate of egg contents was 6.6% in Assiut and 3.3% in Qena governorate. Regarding the sources of chicken’s infection in the farms, the study of Wai et al. [6] proved existence of *H. pullorum* in 17.5% of the house flies and 30% of the house floors in the farms of Malaysia. Moreover, Ceelen et al. [7] isolated *H. pullorum* from farmers’ boots which is regarded as another mechanical source of infection. Concurrent presence of *Helicobacter* and *Campylobacter* species in cats has been reported [28] and water contamination with *Helicobacter* organisms was also demonstrated [29].

**Pathogenesis**

After infection of the host with *H. pullorum*, the bacterium adheres to the microvilli of the intestinal epithelial cells via flagellae for colonization and invasion. Consequently, this invasion induces cellular damage, debris and oedema [30]. In addition, adhesion of the pathogen to the intestinal surface stimulates the production and release of inflammatory substances like IL-8. The inflammatory process is more triggered through production of cytolethal distending toxin and lipopolysaccharide [7]. It has been reported that infection with *H. pullorum* activates the host’s macrophages and secretion of cytokines (TNF-α, IL-1β, IL-6 and MIP-2) as well as production of nitric oxide in murine macrophages [31]. However, Yanagisawa et al. [32] depicted that *H. pullorum* infected human hepatocytes and bile duct and colon epithelial cells displayed increased expression of matrix metalloproteinases 2, 7 and 9 which help in degradation of extracellular matrix and allowing the pathogen to interact with host cells.

**Incidence of infection**

The incidence of *H. pullorum* in poultry and human in different countries is present in Table 2.

**Poultry**

Early study in Switzerland demonstrated *H. pullorum* in the caecal contents of 150 apparently healthy broiler chickens (4%) and in 9 out of 18 caeci of layers with vibrionic hepatitis [33]. Also, in broiler chickens with *Campylobacters* and *Archobacters*, *H. pullorum* was identified from 9 out of 15 frozen cecum (60%) and 9 out of 15 fresh carcasses (60%) [34]. The molecular identification results using Polymerase Chain Reaction (PCR) demonstrated presence of *H. pullorum* in 33.6% of the caecum and in 4.6% of the liver from 110 examined broiler chickens in Belgium [35]. In Italy, Zanoni et al. [36] investigated presence of *H. pullorum* in the caecal contents of 60 chickens that representing 9 broiler and 6 laying chickens’ farms. On the other hand, PCR results showed that 42 out of 55 animals (76.4%) and 11 farms of turkeys were positive for *H. pullorum* [37].

| Sources of *H. pullorum* infection to human | Reference |
|--------------------------------------------|-----------|
| All avian species                          | Andersen [21] |
| The intestinal tract of poultry            | Stanley et al. [2], Ceelen et al. [7], Fox et al. [22] |
| Surface contamination of the chicken       | Ceelen et al. [23] |
| carcasses by the caecal contents           |           |
| Contamination of hands during handling of  | Stanley et al. [2], Atabay et al. [5] |
| the processed chicken carcasses            |           |
| Chicken meat                               | Borges et al. [10], On et al. [24], Gibson et al. [25], Miller et al. [26], González et al. [27] |
| Table eggs                                 | Abdel Hameed and Sender [4] |
| House flies and house floors               | Wai et al. [6] |
| Farmers’ boots                             | Ceelen et al. [7] |
| Cats                                       | Shen et al. [28] |
| Water contamination                        | Azevedo et al. [29] |

Table 1. Sources of *H. pullorum* infection to human.
higher prevalence rate (61%) of *H. pullorum* compared to birds reared in conventional (84%) and organic (97%) farms [38]. The work undertaken by Wai et al. [39] in Selangor and Malaysia identified *H. pullorum* from broiler chickens with 24.72% prevalence rate, where 12.36% of chickens showed concomitant infection with *Campylobacter*. Recently, the same author recognized *H. pullorum* in 51% of caeca of 100 chickens collected from processing sites or markets [6]. In Marmara region of Turkey, *H. pullorum* incidence rate was 55.21% after testing of 12 broiler chicken flocks [40]. Iranian study of Shahram et al. [20] showed that out 120 diarrheic broiler chicken, *H. pullorum* prevalence rates were 7.5% (intestinal swabs), 5% (liver) and 2.5% (thigh meat). However, higher prevalence rate (61%) of *H. pullorum* was also detected in Iran from 100 caecal samples of broiler chickens [41]. The highest incidence of *H. pullorum* in chickens were recorded in many countries where it ranged from 60% in the UK [42] to 78.3% in Czech Republic [43] and 100% in Italy [10] and France [44]. In Egypt, few researches have been conducted to detect the prevalence of *H. pullorum* among different types of living poultry as well as poultry products. A big study has been done in Assuit Province, where 1800 samples were collected from cloacal swabs, caecal contents and liver of chickens, turkeys and ducks’ flocks [45]. The results revealed identification of 100 isolates of *H. pullorum* from chickens with a percentage of 39.33%. Although the main niche for colonization of *H. pullorum* is the intestine especially the caecum, but Hassan et al. [16] proved presence of the pathogen also in the liver tissues of the birds. Moreover, the study of Hassan et al. [46] demonstrated that out of 900 cloacal, caecal and liver tissues of broiler chickens, the incidence rate of *H. pullorum* was 39.33% using species-specific 16S rRNA PCR. Experimentally inoculated broilers with *H. pullorum* elicited 33.3% mortalities with signs of diarrhea, retardation of growth with poor conversion rate and the pathogen was re-isolated from the caecum, liver, yolk sac and air-sacs of dead and sacrificed chickens [46].

### Table 2. Incidence of *H. pullorum* in poultry and human.

| Country              | Findings                                                                 | Reference                     |
|----------------------|--------------------------------------------------------------------------|-------------------------------|
| Switzerland          | *H. pullorum* was detected in the caecal contents of 150 apparently healthy broiler chickens (4%) and in 9 out of 18 caeci of layers | Burnens et al. [33]           |
| Belgium              | *H. pullorum* was molecularly identified in 33.6% of the caecum and in 4.6% of the liver from 110 examined broiler chickens | Ceelen et al. [35]           |
|                      | *H. pullorum* was detected in the clinically healthy persons and the patients with gastroenteritis in percentages of 4% and 4.3%, respectively | Ceelen et al. [7]             |
| Italy                | *H. pullorum* was present in the caecal contents of 60 chickens that representing 9 broiler and 6 laying chickens’ farms | Zanoni et al. [36]           |
|                      | *H. pullorum* was isolated from chickens in incidence of 100%             | Borges et al. [10]            |
| Malaysia             | *H. pullorum* was isolated from broiler chickens with 24.72% prevalence rate, where 12.36% of chickens showed concomitant infection with *Campylobacter* | Wai et al. [39]               |
|                      | *H. pullorum* in 51% of caeca of 100 chickens collected from processing sites or markets | Wai et al. [6]                |
| Turkey               | *H. pullorum* incidence rate was 55.21% after testing of 12 broiler chicken flocks | Beren and Seyyal [40]         |
| Iran                 | Out 120 diarrheic broiler chicken, *H. pullorum* prevalence rates were 7.5% (intestinal swabs), 5% (liver) and 2.5% (thigh meat) | Shahram et al. [20]           |
|                      | The prevalence rate of *H. pullorum* was 61% from 100 caecal samples of broiler chickens | Jamshidi et al. [41]          |
|                      | Six positive cases of *H. pullorum* was detected in 100 stool samples of patients with gastroenteritis | Shahram et al. [20]           |
| United Kingdom       | *H. pullorum* was isolated from chickens in incidence of 60%              | Sergeant et al. [42]          |
| Czech Republic       | *H. pullorum* was isolated from chickens in incidence of 78.3%             | Svobodova and Boribova [43]   |
| France               | *H. pullorum* was isolated from chickens in incidence of 100%              | Pilon et al. [44]             |
| Egypt                | Identification of 100 isolates of *H. pullorum* from cloacal swabs, caecal contents and liver of chickens with a percentage of 39.33% | Mohamed et al. [45]           |
|                      | Out of 900 cloacal, caecal and liver tissues of broiler chickens, the incidence rate of *H. pullorum* was 39.33% | Hassan et al. [46]            |
|                      | Experimental infection with *H. pullorum* in chickens elicited 33.3% mortalities with signs of diarrhea, retardation of growth with poor conversion rate and the pathogen was re-isolated from the caecum, liver, yolk sac and air-sacs of dead and sacrificed chickens | |

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Human

Diarrhea caused by infectious agent is a major cause of worldwide morbidity and mortality in human, especially in children [47]. There are some reports suggesting that *H. pullorum* is a major pathogen of human. Early, *H. pullorum* was first isolated from the stool of a male patient with diarrhea and elevated liver enzymes [48]. Later on, this pathogen was discovered from faeces of diarrheic patients, 3 months after the onset of symptoms [49]. Infection of human with *H. pullorum* is not only associated with gastroenteritis and diarrhea, but also with gall bladder and liver diseases [22]. In addition, *H. pullorum* was isolated from 35 year old male suffering from bacteraemia, abdominal pain and profound diarrhea [50]. In 2005, *H. pullorum* was detected in the clinically healthy Belgium persons and the patients with gastroenteritis in percentages of 4% and 4.3%, respectively [7]. They concluded that presence of *H. pullorum* in the stool of apparently healthy individuals may indicate that this bacterium is harmless normal inhabitant in the intestine or it proliferates after consumption of contaminated food. They also assumed that certain unknown predisposing factors may change non-pathogenic normal intestinal *H. pullorum* to highly virulent pathogenic ones. *H. pullorum* has also been identified by PCR in humans with inflammatory bowel disease [41,51], viral hepatitis C [52-54], cholecystitis [55,56] and hepatocellular carcinoma [57,58]. Another study of Shahram et al. [20] recognized 6 positive cases of *H. pullorum* from 100 stool samples of patients with gastroenteritis in Ardabil province, Iran. It was also found that *H. pullorum* may have an important role in Crohn's disease caused by *Mycobacterium paratuberculosis* in inflammatory bowel disease [59,60].

Treatment

Unfortunately, there is no recommended groups of drugs for treatment of *H. pullorum* infection. The sensitivity or resistance of *H. pullorum* isolates to different antimicrobials have been studied with variable results. The *in-vitro* resistance of avian *H. pullorum* isolates to nalidixic acid revealed percentages of 6% [24] and 26% [5]. Moreover, resistance of *H. pullorum* to cephalothin and cefoperazone was also recorded [2, 24,36]. Recently, tetracycline resistance of *H. pullorum* mutant strain was recorded [10]. Conversely, *H. pullorum* was found to be susceptible to polymyxin B [5]. Moreover, human strains of *H. pullorum* displayed sensitivity to aminoglycosides, third-generation cephalosporins, β-lactams and doxycycline [50]. In Upper Egypt, high incidence of avian *H. pullorum* resistance to ciprofloxacin, gentamicin and erythromycin followed by tetracycline were observed [45,46]. Nevertheless, the same studies revealed high sensitivity of the pathogen to ampicillin and/or colistin sulfate suggesting them as drugs of choice for treatment of infection in chickens. The study of Abdel Hameed and Sender [4] indicated that *H. pullorum* isolated from chickens’ eggs were resistant to ampicillin, ceftriaxone and sulphamethoxazole trimethoprim *in-vitro*.

Conclusion

It has been considered that *H. pullorum* is an emerging pathogen of potential zoonotic importance for both human and animals. Little is known about this bacterium infection. So, more extensive attention and studies should be carried out to increase the knowledge and information about *H. pullorum* prevalence, infectivity and control measures. The closed-housing system with good biosecurity, management and husbandry practices could reduce and control the presence of *H. pullorum* in the farms. It is important to focus on the methods of control of this pathogen at the farm level till retailing. These data will have a public health importance in relation to reducing human exposure associated with the handling and consumption of contaminated processed chicken’s meat.

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Corresponding author
Wafaa A. Abd El-Ghany
Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University Giza, Egypt (12211)
Tel: +02 01224407992
Email: wafaa.ghany@yahoo.com

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