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A REVIEW: EXPERIMENTAL ANIMAL MODEL OF HELICOBACTER PYLORI IN 2018-2023

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Abstract

H. pylori is a gram-negative bacterium that has colonized the stomachs of approximately 50% of the human population worldwide. H. pylori is a major pathogen of gastrointestinal diseases, including gastritis, gastric ulcers, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT). To study the pathogenesis, prevention and therapy of H. pylori infection, ideal, safe and stable animal model experiments are needed. This text summarizes several important aspects in successfully creating experimental animal models of H. pylori. The method used is literature study through two electronic databases, namely Sciencedirect and Pub Med. A total of 50 articles were used for full text assessment according to inclusion and exclusion criteria. The results showed that C57BL/6 and BALB/c animal models were able to produce gastritis, metaplasia and hyperplasia and rarely produced cancer, while Mongolian gerbils and transgenic mice were considered more susceptible to creating cancer models. The strains commonly used in research on H. pylori infection are CagA-positive SS1 and PMSS1.

Keywords: Helicobacter pylori, In vivo, Animal Model

Abstrak

H. pylori adalah bakteri gram negatif yang telah mengkolonisasi perut sekitar 50% dari populasi manusia di seluruh dunia. H. pylori merupakan pathogen utama penyakit gastrointestinal, termasuk gastritis, tukak lambung adenokarsinoma lambung, dan jaringan limfoid terkait mukosa lambung (MALT). Untuk memepelajari patogenesis, pencegahan dan terapi terhadap infeksi H. pylori, maka diperlukan eksperimen model hewan yang ideal, aman dan stabil. Teks ini merangkum beberapa aspek penting dalam keberhasilan membuat model hewan coba H. pylori. Metode yang digunakan adalah studi literatur melalui dua basis data elektronik yaitu Sciencedirect dan Pub Med. Sebanyak 50 artikel digunakan untuk penilaian teks lengkap sesuai dengan kriteria inklusi dan eksklusi. Hasil menunjukkan, model hewan C57BL/6 dan BALB/c mampu menghasilkan gastritis, metaplasia dan hyperplasia serta jarang menghasilkan kanker, sedangkan Mongolia gerbil dan tikus transgenic dianggap lebih rentan untuk membuat model kanker. Strain yang biasa digunakan dalam penelitian infeksi H. pylori adalah SS1 dan PMSS1 dengan CagA-positif.

***Kata Kunci**: Helicobacter pylori, In vivo, Animal Model*

1. INTRODUCTION

H. pylori is a spiral-shaped, microaerophilic, extracellular Gram-negative bacterium that has colonized the stomachs of approximately 50% of the human population worldwide, making it the most common human pathogen worldwide (Venerito et al., 2018). H. pylori infection initially causes gastric inflammation and over time the development of gastrointestinal diseases, including gastritis, peptic ulcers, and chronic gastritis (Li et al., 2023), intestinal metaplasia, dysplasia (Ray et al., 2021) duodenal and gastric ulcers, gastric adenocarcinoma, and mucosa- associated lymphoid tissue (MALT). (Henriques et al., 2020). This bacterium colonizes the human stomach causing severe mucosal inflammation and if not treated, the infection persists throughout life (Chew et al., 2017). Epidemiological data indicate that transmission of infection occurs via oral, fecaloral, or iatrogenic routes. H. pylori can be cultured from the vomit, feces, and saliva of infected individuals, indicating potential for transmission. In addition, water can be a potential source of infection transmission (Kayali et al., 2018). Several H. pylori proteins have been identified as immunogenic

in preclinical models, including Urease B (UreB), Vacuolating toxin A (VacA), H. pylori adhesion A (HpaA), neutrophil activating protein A (NapA), outer membrane protein (Omp), cytotoxin-associated antigen (CagA), OipA (Ghasemi et al., 2018). One of the factors, CagA plays an important role in the inflammatory response. CagA secreted by H. pylori is responsible for activating critical pathways such as NF-kB, Akt, src/MEK/ERK (Hayashi et al., 2017). In fact, virulence factors can trigger different gene expression cascades, resulting in the activation of proinflammatory transcription factors such as NF-kB, AP1, and the production of cytokines to facilitate gastric damage, such as to facilitate the release of cytokines including TNF-α, IL6, IL8, IFN-γ, TGF-β, IL-2, IL-6, IL-17 and COX2 (Zhang et al., 2016).

To study the pathogenesis, prevention and therapy of H. pylori infection, ideal, safe and stable animal model experiments are needed. An ideal animal model must meet the following conditions: (1) The model develops a disease that is similar to the same condition in humans; (2) Animals are abundant and easy to care for; and (3) The model is simple and provides a high success rate, and easily guarantees animal survival (Wei et al., 2019). H. pylori infections have developed in mice, gerbils, cats, and dogs, and in gnotobiotic pigs. Current understanding is that the nature of H. pylori infection is highly variable and depends on the organism and the genetic background of the host (Mishra et al., 2019). Therefore, in this text we summarize several important aspects in successfully establishing experimental animal models of H. pylori.

2. RESEARCH METHOD

2.1 Search Methods

A comprehensive and systematic literature search was carried out, summarizing methods for creating experimental animal models related to H. pylori infection. The literature search was carried out in March 2023–September 2023 through two electronic databases, namely Sciencedirect and Pub Med. The search keywords used in the literature search were "Helicobacter pylori" AND "Animal Model" OR "In Vivo". The articles obtained were then filtered based on inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria are created so that the references included in the review are not too broad and focus on research that is in line with the objectives of this study. The inclusion criteria for searching for references in this study are: (1) the reference is original experimental research;(2) the reference discusses Helicobacter pylori infection; (3) the reference uses an experimental animal model (in vivo); (4) the reference was published in the last 5 years; (5) use English. References that do not match the inclusion criteria are considered to be exclusion criteria. Next, all references are screened.

2.2 Data Extraction and Analysis

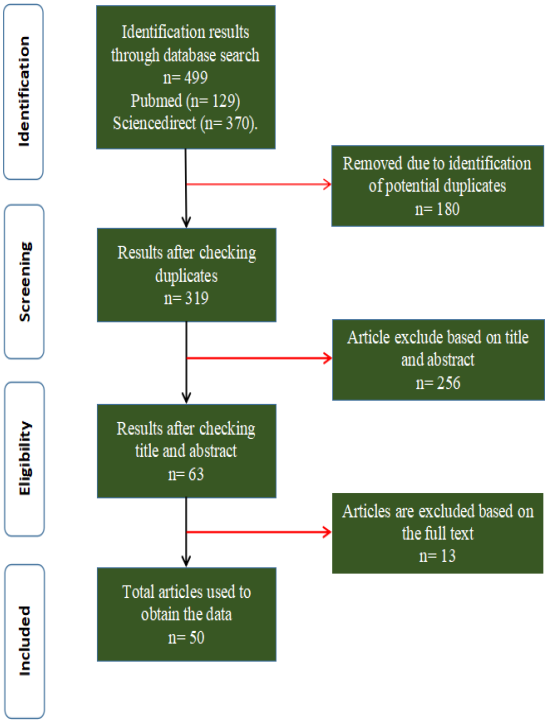

Figure 1. Literature selection flow diagram

Screening is carried out by identifying duplications and due diligence through the title and abstract. Eligible references were retrieved for full text evaluation. Furthermore, PRISMA was created to assist authors in developing systematic reviews by creating a Process of identifying eligible studies chart (Figure 1). A final reference suitable for use in this study would be to identify methods for creating experimental animal models with H. pylori infection.

3. RESULTS AND DISCUSSION

Based on the results of reference searches through electronic databases, 499 articles were found that matched the keywords, 370 articles came from the Sciencedirect database and 129 articles came from the Pubmed database. Of the 499 articles, 180 articles had the potential to be duplicates, leaving 319 articles. Of the 319 articles, 256 articles did not meet the criteria, leaving 63 articles. The remaining 63 articles were then evaluated for full text eligibility and it was found that 14 articles were deemed not to meet the criteria, resulting in a total of 50 articles deemed relevant and suitable for use (Figure 1). Furthermore, these 50 articles are summarized in table (1-3) to provide information regarding the creation of experimental animal models related to H. pylori. H. pylori adapts well to the human stomach. H. pylori adheres primarily to mucus-secreting gastric epithelial cells on the luminal surface, while colonization usually begins in the human antrum, H. pylori primarily resides in the most proximal part of the murine columnar stomach, close to the squamo columnar junction, and progresses progressively through the corpus (Alpizar et al., 2020). It is difficult to determine the pathogenesis of

H. pylori infection and the immune response produced by this pathogen. Therefore, much effort has been made to identify suitable animal models to understand the natural history of H. pylori infection and its immune response (Di et al., 2020). In table 1-3, it is known that animal models that have been used in research on H. pylori infection in the 2018- 2023 period include C57BL/6, BALB/c, Kunming, Mongolian gerbil, Wistar, Hamster, Guinea Pig and transgenic mice. C57BL/6, BALB/c and Mongolian gerbil are the top 3 in the use of experimental animal models related to H. pylori.

3.1 Animal Model C57BL/6

In this text, research on H. pylori infection mostly uses mice, including C57BL/6 and BalB/C. The use of C57BL/6 in H. pylori infection research using the SS1 strain showed a significant increase in bacterial colonization of the gastric mucosa and caused inflammation. Inflammation is generally characterized by infiltration of inflammatory cells (mainly lymphocytes, macrophages, and some neutrophils) (Khan et al, 2023; Park et al., 2020). It is known that increased inflammation and ulceration as well as stomach damage occur at 4 weeks post-infection (Hong et al., 2018) after which the inflammation spreads globally throughout the stomach including the corpus and antrum and causes chronic superficial gastritis at 6-16 weeks post- infection (Morningstar et al., 2022; Luo et al., 2018). Furthermore, the situation worsens into atrophic gastritis and metaplasia at 25-36 weeks post-infection (Alpizar et al., 2020; Park et al, 2021). Based on several studies (Table 1), it is known that H. pylori infection using the SS1 strain increases CagA, IgG, TNF-α, NF-ƙB, NOD-1, IL-6, IL-1β and IL-8 (Saha et al., 2022; Shi et al., 2022; Song et al., 2022; Le et al., 2022) as well as phosphorylation of p38 MAPK, JNK,

ERK1/2 and p65 in gastric tissue (Park et al., 2020; Park et al., 2018). Another strain, namely ATCC 43504, produced an increase in IgG levels of 93% at 2 weeks post- infection and eosinophilic inflammation occurred which was characterized by an increase in eosinophilic granules in most layers of the lamina propria at 4 weeks post-infection (Hong et al., 2018). Strain 26695 produces increased expression of VacA, IL-1β, IgA, IgG and IgM for 2 weeks (Chen et al., 2018), while strain NCTC 11.637 is known to increase oxidative stress by increasing the expression of the inflammatory factors COX2, IL6, and TNF-α for 12 weeks (Zhang et al., 2022). An excessive and continuous increase in inflammatory factors will be responsible for linking inflammation to the severity of disease, including cancer.

3.2 Animal Model BALB/c

The BALB/c strain is another widely used mouse test, including H. pylori infections. BALB/c is a lab-bred albino, immunodeficient house mouse breed that was initially established in 1913. Reasons for using BALB/c include easy reproduction, small weight differences between males and females, and extreme sensitivity (Lin et al., 2020). Table 1, Vaillant, et al (2021) shows H. pylori infection using Balb/C with H. pylori strain 49 (Hp49) showing an increase in eosinophils on the second day post- infection. Activation of eosinophils in the blood is a prerequisite for their recruitment into inflamed tissue (Johansson, 2014). In BALB/c infected with H. pylori strain 26695 (ATCC 700392) for 14-25 days, it causes severe inflammatory cell infiltration, increased levels of IL-33, ST-2 (Kuo et al., 2019), TNF-α and COX-2 (Chen et al., 2019). Another BALB/c study using a

mucosal injury and erosion, atrophic gastritis

and superficial gastritis occurred within 2-4 weeks post-infection (Hong et al., 2018; Luo et al., 2018; Shi et al., 2022) and 25-30 weeks post-infection successfully develops into metaplasia (Alpizar et al., 2020). BALB/c using strain BCRC15415 at 2 weeks post-infection can cause chronic inflammation and hyperplasia (Chen et al., 2022). Because the murine stomach may contain some other bacteria that can influence the pathogenicity of H. pylori infection, some studies have used transgenic or knockout animal models Table 2. This animal model was used to explore the different strain of H. pylori, namely NCTC 11638, for 7-30 days showed severe tissue damage, infiltration of inflammatory cells, the presence of a large number of blood vessels and small to medium sized erosions which were increasing both in number and size reaching the highest level on day 30 post-infection (Sossa et al., 2022). The use of strain BCRC15415 in BALB/c for 14 days of infection resulted in chronic inflammation and hyperplasia, significantly increased expression of TNF-α, IL-6, and IL-1β (Chen et al., 2022), whereas long- term infection of strain SS1 with single dose shows moderate chronic gastritis which increases the titer of IgG, IgA specific UreA or UreB until the 17th week (Liu et al., 2020).

C57BL/6 is one of the most widely used substrains because it is more susceptible to various diseases and infections (Eisfeld et al.., 2019; Kang et al., 2019). The differences between BALB/c and C57BL/6 strains based on gut microbiota are that the genetics of C57BL/6 mice appear to be more pure, so the gut microbiota is more stable, whereas BALB/c have a highly variable gut microbiota, so BALB/c mice may be a more similar model to humans (Guo et al., 2022; De Filippis et al., 2019). Apart from that, another difference in the field of immunology is the immune response. It is known that BALB/c predominantly shows a Th2 response, while C57BL/6 mice show a dominant Th1 response to the same stimuli (Amalia et al., 2022). In H. pylori infection, Th1 tends to be in the chronic infection model, while Th1 is in the acute infection model (Kuo et al., 2019). In the C57BL/6 study using the H. pylori SS1 strain, gastric

effect of genetic composition on important expression in both the inflammatory response and H pylori-induced carcinogenesis (Peng et al., 2019). Some of these mouse models, such as insulin-gastrin (INS-GAS) (Stair et al., 2023), TFF1-KO

(Shao et al., 2018; Soutto et al., 2021), mTOR KO (Feng et al., 2020), K19-IL11

(Buzzeli et al., 2018), Deficiency l17−/− (Dixon et al., 2022) and

Krt19Cre/Ert

/Cdk1flox/flox (Zhu et al., 2023).

Table 1. *Helicobacter pylori* Infection in C57BL and BALB/c

No	Animal Model	Gender	Age	Strain *H. pylori*	*H. pylori* dosage	Inoculation Duration	Colonization Duration	Inspection Method *H. pylori*	Ref
1	C57BL/6	Female	5-6 weeks	SS1	10^8 CFU in 0.2 mL of 0.85% NaCl	a single dose	3-30 weeks	Immunohistochemistry	1
2	C57BL/6	Male	4 weeks	26695	1×10^9 CFU	Every other day for a total of three doses	2 weeks	ELISA	11
3	C57BL/6	Male	Unknown	SS1	1×10^9 CFU in 0.5 mL broth by intragastrically	Three times at 3-day intervals	4 weeks	Histologi and ELISA	31
4	C57BL/6	Female	Weeks	43504	0.2 mL of H. *pylori* 2 x 10^8 CFU by intragastric gavages	Three times per week for 4 weeks	2-4 weeks	Immunohistochemistry and ELISA	30
5	C57BL/6	Male	8 weeks	SS1	10^8 CFU/mouse/inoculation by orally	On three alternative day	6 weeks	Histology nd ELISA	38
6	C57BL/6	Male	8 weeks	SS1	0.2 mL 10^8 CFU of H. *pylori* by gastric intra-gavage	Every alternate day for a week	8 weeks	Histology and ELISA	65
7	C57BL/6	Male	6 weeks	SS1	0.5 ml Brucella broth of H. *pylori* 3×10^8 CFU/mL	Every mouse was intubated five times	2 weeks	Immunohistochemistry and ELISA	67
8	C57BL/6	Male	4 weeks	SS1	1×10^9 CFU/mL/mouse by orally	3 times at 2-day intervals.	8 weeks	Histology and ELISA	61
9	C57BL/6	Female	4-5 weeks	SS1	1×10^7 CFU in .5 ml Brucella Broth by oral gavage	Single dose	4-16 weeks	Histology and RT-PCR	54
10	C57BL/6	Male	4 weeks	SS1	5.0×10^9 CFU/mL	Four times at 2-day intervals	5 weeks	Histology nd ELISA	68
11	C57BL/6	Male	4 weeks	SS1	200 µL of Columbia broth containing 10^8 CFU by orally	Daily for eight weeks	5 days	Histologi dan qPCR	41
12	C57BL/6	Female	5 weeks	PMSS 1	1×10^9 CFU/0.2 mL/mouse by orally	At 2-day intervals 4 times	5 weeks	Histology nd ELISA	78
13	C57BL/6	Male	6-8 weeks	NCTC 11637	3×10^8 CFU by orogastrically	Twice a day for 3 consecutive days	12 weeks	immunohistochemistry and RT-PCR	89
14	C57BL/6	Female	4 weeks	SS1	100 µL of Brucella broth containing 5×10^8 CFU/ ml by oral gavage	Single dose	2-4 weeks	Immunohistochemistry, CBA and ELISA	47
15	C57BL/6	Male	4 weeks	SS1	5×10^9 CFU/0.2 mL/mouse by orally	Three times at 2- day intervals	13 weeks	Histology, ELISA, immunohistochemistry and RT-PCR	60

No	Animal Model	Gender	Age	Strain *H. pylori*	*H. pylori* dosage	Inoculation Duration	Colonization Duration	Inspection Method *H. pylori*	Ref
16	C57BL/6	Unknown	5 weeks	SS1	0.3 ml Trypticase soy broth containing 1×10^9 CFUs/ml	Total fourth times within a week	24-36 weeks	Histology and RT-PCR	59
17	C57BL/6	Female	6-8 weeks	SS1	5×10^8 CFU by orally	Thrice in 2-day intervals	15-75 days	ELISA and qPCR	23
18	C57BL/6	Male	4-5 weeks	Isolat Klinis	0.3 mL H. pylori suspension in PBS 1×10^9 by orally gavaged	Total 3 times for 9 days (Once daily with 1 day off)	2 weeks	RT- PCR and Bioinformatic	42
19	C57BL/6	Male	5-6 weeks	SS1	0.2 mL suspension of 1×10^{10} CFU/mL by oral gavage	Twice with two days interval	5 weeks	Histology, and PCR	29
20	C57BL/6	Male	6–8 weeks	PMSS 1	2×10^7 CFU by intragastrically	Three times at 2 day intervals	4 weeks	Western blotting dan imunofluorence	64
21	C57BL/6	Female	Unknown	SS1	300 µl PBS containing 1×10^9 CFU/ ml by intragastrically	On alternate days for five times	8 weeks	Histology, ELISA and cytometri	87
22	C57BL/6	Unknown	Unknown	PMSS 1	$1x10^9$ CFU by oral gavage	Single dose	1-2 weeks	Histology and qRT-PCR	8
23	BALB/c	Female	6-8 weeks	Hp49	5×10^8 CFU in 200 µl of BHI by oral gavage	Twice with two days interval	8 weeks	Quantification of Hp and qRT-PCR	82
24	BALB/c	Female	3 weeks	SS1	3×10^{10} CFU in 0.2 ml by orally	Single dose	11-17 weeks	Histology, ELISA	45
25	BALB/c	Male	6 weeks	26695	1×10^8 CFU by intragastric gavage	Once every two days for a total of six injections	2 days	Immunohistochemistry , ELISA dan qRT-PCR	40
26	BALB/c	Male	Adult	NCTC 11638	300 µl suspension 1–5 $\times 10^8$ CFU/ml by intragastrically gavage	Every 3 days for 3, 15, or 30 days	7-30 days	Histology	70
27	BALB/c	Male	5 weeks 2	BCRC 15415	1×10^9 CFU/mL	2 days (at days 0 and 2)	2 weeks	Histology and qRT-PCR	14
28	BALB/c	Male	6 weeks	ATCC 700392	1×10^8 by intragastric gavage	Total of 6 administrations (at days 8, 10, 12, 14, 16, and 18).	1 weeks	Histology and Immunohistochemistry	13
29	BALB/c	Unknown	6-8 weeks	SS1	1×10^8 CFU by orally	Three times with two-day intervals	40 days	ELISA	53
30	BALB/c	Female	6 weeks	SS1	200 µl containing 5×10^7 CFU by orally	Single dose	2 and 6 weeks	Quantification of Hp and ELISA	90

No	Animal Model	Gender	Age	Strain *H. pylori*	*H. pylori* dosage	Inoculation Duration	Colonization Duration	Inspection Method *H. pylori*	Ref
31	BALB/c	Female	5 weeks	SS1	10^8 CFU in 0.1 ml Brucella Broth by intragastrically	At 2-day intervals with three doses	After the last HP dose	ELISA and qRT-PCR	56
32	BALB/c	Male	6-12 weeks	NCTC 11638	20 µl suspension 1–5x10^8 CFU/ml by orotracheal instillation	Once per day throughout 3 days	3-30 days	Histology	69

3.3 Transgenic Animal Models

Furthermore, the use of transgenic mouse models is very beneficial for research. This is because in the process animal models can be made specifically and more quickly to determine certain effects on the pathogenesis of H. pylori infection. Because the murine stomach may contain some other bacteria that can influence the pathogenicity of H. pylori infection, some studies have used transgenic or knockout animal models Table 2. This animal model was used to explore the effect of genetic composition on important expression in both the inflammatory response and H pylori-induced carcinogenesis (Peng et al., 2019). Some of these mouse models, such as insulin-gastrin (INS-GAS) (Stair et al., 2023), TFF1-KO (Shao et al., 2018; Soutto et al., 2021), mTOR KO (Feng et al., 2020), K19-IL11 (Buzzeli et al., 2018), Deficiency l17−/− (Dixon et al., 2022) and Krt19Cre/Ert/Cdk1flox/flox (Zhu et al., 2023). Table 2, as in the study by Buzzeli, et al (2018) which used a single dose of SS1 and a mouse transgenic model (K19-IL11Tg) namely stomach-specific IL-11 overexpression was created to investigate the effect of increasing IL-11 levels on gastric epithelial homeostasis. The results showed that K19- IL11Tg infected mice showed more severe immunopathology compared with infected WT mice with inflammation, atrophy, epithelial hyperplasia and metaplasia consistently increasing over time. Approximately 20% of 52-week-old K19- IL11Tg mice developed extensive and severe hyperplastic lesions in the corpus epithelium suggesting that K19-IL11Tg mice have increased susceptibility to H. pylori SS1- associated pathology with a role for IL-11 as a driver of inflammatory precancerous disease (Howlett et al., 2009; Menheniott et al., 2016; Nakayama et al., 2007). Furthermore, the use of the PMSS1 strain with a different transgenic model, namely INS-GAS, aims to overexpress pancreatic gastrin (Hayakawa et al., 2013; Whary et al., 2014). In this model, it is documented that the presence of gastritis, epithelial defects, oxyntic atrophy, epithelial hyperplasia, pseudopyloric metaplasia, and dysplasia also reduces the number of erythrocytes, hematocrit and HB which leads to mild- moderate anemia and is followed by an increase in IL-1β, IL-10, and IFN. -γ at 27- 29 weeks post-infection (Strair et al., 2023). TFF1 is a small secreted protein that protects the integrity of the gastric mucosa and promotes its repair after injury (Hu et al., 2018), so in another study using a TFF1-KO transgenic model aimed to investigate the role of TFF1 in suppressing NF-κB and STAT3-mediated activation. by H. pylori PMSS1. Results revealed that H. pylori promoted the activation of NF-κB and STAT3 pro-inflammatory signaling (Tran et al., 2017) and promoted the expression of several inflammatory target genes (Sue et al., 2015). However, TFF1 was able to reverse this effect and inhibit NF-κB-mediated STAT3 activation as well as abrogate the induction of IL-6, VEGF-ÿ, IL-17 and IL-23 by H. pylori infection (Soutto et al., 2021).

Table 2. *Helicobacter pylori* Infection in Transgenic

No	Model Transgenic	Strain *H. pylori*	*H. pylori* dosage	Inoculation Duration	Colonization Duration	Inspection Method *H. pylori*	Ref
1.	mTOR KO	NCTC 11637	3×10^8 CFU by oralgastrically	Twice at a 1-day interval	2-7 weeks	Immunohistochemistry, ELISA and qRT-PCR	22
2.	K19-IL11	SS1	10^7 H. pylori SS1 by intragastrically	Single Dose	12-52 weeks	Immunohistochemistry and qRT-PCR	7
3.	INS-GAS	PMSS1	$1x10^8$CFU by gastric gavage	On alternating days for a total of three doses	12-29 weeks	Immunohistochemistry, ELISA, UIBC	72
4.	TFF1-KO	PMSS1	1×10^9 CFU in 0.5 ml of Brucella broth by oral gavage	Three consecutive days	3 weeks	Histology, imunofuoresensi and qRT-PCR	71
5.	TFF1-KO	PMSS1	10^9 CFU/mouse	Single dose	2 weeks and 6 month	qRT-PCR	66
6.	Deficiency l17−/−	PMSS1	5×10^8 CFU or $1x10^9$ in 0.5 ml of Brucella broth by orogastrically	Each dose was given twice, two days apart	1-3 months	Histology and qRT-PCR	18
7.	$Krt19^{Cre/Ert}$ /$Cdk1^{flox/flox}$	PMSS1	10^9 CFU PMSS1	Once per day throughout 14 days	1-2 months	Immunohistochemistry and qRT-PCR	91

3.4. Other Animal Models

Another experimental animal model that is considered a useful model for H. pylori infection is the Mongolian gerbil (MG) Table 3. MG can be used as hosts for long-term H. pylori infections, because it is known that the MG model of H. pylori colonization shows stable results (Watanabe et al., 1998) and the pathological changes in the gastric mucosa after infection are very similar to those that occur in humans (Jin et al., 2008). Therefore, MG has long been a widely accepted animal model for studying and analyzing gastric mucosal diseases caused by H. pylori infection. In this text, the number of studies using MG is less than the number of studies using mice. In a MG model study using H. pylori strain 26695 (ATCC 700392), it produced moderate chronic active gastritis at 8 weeks, mild atrophy appeared at 12-26 weeks followed by an increase in IgG titers (Mishra et al., 2019), whereas in other studies with animal models and similar strains resulting in the development of mild gastritis that progresses to moderate and severe with atrophy over 6-18 months and is followed by increased expression of miRNA-146a and miRNA-155 (Marquez et al., 2018).

Furthermore, the MG model with a different strain, namely 60190 single dose, resulted in an increase in the number of neutrophils, eosinophils, lymphocytes, IL- 1β, IL-6, IL-4 and IFN-γ at 8 weeks post- infection (Kim et al., 2019), strain 7.13 single dose showed metaplasia and gastric atrophy accompanied by increased moderate to severe PMN infiltration in the corpus and antrum at 10 weeks post- infection (Su et al., 2022), while strain NCTC11637 succeeded in producing superficial gastritis, atrophic gastritis and gastric ulcers in 52 weeks post infection (Chen et al., 2018).

Further in this text we also find several other animal models used in H. pylori infection, including Kunming, Wistar and Guinea Pig. Kunming (KM) Table 3 is considered the mouse strain of choice for laboratory research, including vaccination and drug investigations. These mice are descendants of Swiss mice which have been bred into various inbred lines in various parts of the world. Disease resistance, adaptability, high reproductive rate, and high survival rate differentiate them from Swiss mice (Mei et al., 2022).

Table 3. *Helicobacter pylori* Infection in Other Animal Models

No	Animal Model	Gender	Age	Strain *H. pylori*	*H. pylori* dosage	Inoculation Duration	Colonization Duration	Inspection Method *H. pylori*	Ref
1	Mongolian gerbils	Male	6 weeks	NCTC 11637	1 ml suspension 1×10^8 CFU/ml by intragastrically gavage	Once a day for 3 days.	52 weeks	Histology dan Imunohistokimia	12
2	Mongolian gerbils	Female	5-6 weeks old	26695	1.0 ml suspension in Brucella broth of 10^9 CFU/mL by orogastric	Three times daily on days 0, 2, and 4.	4-52 weeks	Histology, dan ELISA	51
3	Mongolian gerbils	Male	8 weeks	26695	6×10^8 CFU/mL by intragastrically	Every day for one week.	3-18 months	Histology, dan qRT-PCR	48
4	Mongolian gerbils	Male	Unknown	VM202-203	1 x 10^9 CFU via oral gavage	2 days (at days 0 and 2)	90 days	Quantification of Hp and Histology	43
5	Mongolian gerbils	Male	5 weeks	7.13	500 µl of brucella broth containing 1×10^8 CFU	Single dose	2 and 10 weeks	Quantification of Hp and Histology	73
6	Mongolian gerbils	Female	4 weeks	60190	1×10^9 CFU in 1 mL of sterile distilled water by orogastrically	Single dose	8 weeks	Imunohistokimia, Hematology and qRT-PCR	39
7	Kunming Mice	Female	6 weeks	Clinically isolated	5×10^8 CFU by intragastrically	Every other day, five times in succession	30-120 days	Histology, Imunohistokimia dan TUNEL	85
8	Kunming Mice	Male and Female	Unknown	43504	0,4 mL suspension $1x10^9$ cfu/mL by intragastrically	Once per day throughout 7 days	5 weeks	Histology	46
9	Syrian golden hamsters	Male	6-8 weeks	P12	1.7×10^8 CFU/ml via intragastric	Single dose	1-6 months	Histology dan Imunohistokimia	75
10	Wistar rats	Male	3 Months	Clinically isolated	1 mL (2.7×10^9 CFU) using gavage	Twice daily with 4 h interval, and for 8 sequential days.	2 weeks	Imunohistokimia, dan qRT-PCR	63
11	Guinea pigs	Male	3 Months		1 ml of Brucella broth suspension 1×10^{10} CFU	per os three times (at two-day intervals	7-28 days	Histology, ELISA dan PCR	24

In Wei *et al* (2019) study, using a Kunming animal model infected with clinical isolates of *H. pylori* succeeded in producing acute gastritis on the 30th day post-infection. Increased TNF-α, IL-8, and IL-1β and Bax, while significantly decreased the expression of Bcl-2, CD4+ and CD8+ until day 120 post-infection, indicating that gastritis caused by *H. pylori* is characterized by impaired immunological function. 90% of mice in the combined group developed chronic atrophic gastritis (CAG) at 90 and 120 days *after H. pylori* infection and colonization.

Another animal model considered suitable is Caviae porcellus (guinea pig), due to the anatomy and physiology of its stomach, which is similar to the human stomach, the ability to produce the human proinflammatory interleukin (IL) 8 homologue, and to develop specific humoral and cellular immune responses against H. pylori (Miszczyk et al., 2014). Gonciarz et al (2019) research using Caviae porcellus with the H. pylori strain CCUG 17874, observed the occurrence of acute infection within 7 days post-infection, while the chronic phase occurred after 28 days which was characterized by an increase in the number of eosinophils and lymphocytes. Additionally, studies using Wistar with clinical isolates of H. pylori have also been well documented. H. pylori infection in Wistar causes metaplasia, apoptosis, necrosis, focal ulceration with submucosal congestion, edema, and mononuclear cell infiltration. Furthermore, there was an increase in the expression of TNF-α and COX-2 in the foveolar epithelium, as well as an increase in lipid peroxidation as evidenced by an increase in MDA concentration (Ragab et al., 2022).

3.5. *Helicobacter pylori* Strains

H. pylori strains contain a virulence determining region known as the cag Pathogenicity Island (cag-PAI) which is important in the pathogenicity of infection (Oliveira et al., 2009). The presence of the cag pathogenicity island (PAI) in H. pylori is associated with increased mucosal inflammation and increased risk of development of gastric cancer or peptic ulcer disease, where CagA and VacA are the main virulence factors H. pylori (Fahimi et al, 2017). Different strains have different genes, so the virulence of various H. pylori strains may be different (Chang et al, 2018). The strains most commonly used in research on H. pylori infection are SS1 and PMSS1. The Sydney strain of H. pylori SS1, is a CagA+ and VacA+ with significant virulence, first obtained from a biopsy of a dyspeptic patient, with a characteristic S shape that adapts to colonizing mice effectively (Amalia et al., 2022).

As has been documented in C57BL/6 mice, the inflammation was exacerbated at 25 and 30 weeks post- infection (Alpizar et al., 2020), whereas in BALB/c mice showed moderate chronic gastritis until week 17 (Liu et al., 2020). Suzuki et al (2022), compared the effects of long-term infection (18 months) with the H. pylori SS1 strain in Mongolian gerbils and C57BL/6 mice. It is known that the response between the two experimental animal models is similar. However, the level of inflammation and disease pathogenesis is lower than in experimental animal models infected with the Japanese strain of H. pylori TN2GF4 which produces gastric adenocarcinoma in 37% of experimental animals (Watanabe et al., 1998). This is because the H. pylori SS1 strain, known to be cagA positive, undergoes recombination in Y cells (Amalia et al., 2022) which disrupts T4SS function and loss of cag PAI function (Morningstar et al., 2022) reduces its tendency to inflammation (Barrozo et al., 2013). PMSS1 is a clinical isolate from a duodenal ulcer patient, first introduced by Arnold et al. Unlike SS1, PMSS1 has a functional cag-PAI that produces the corresponding protein and can inject CagA into human gastric epithelial cells in vitro and is thought to remain functional for at least 16 weeks postinfection (Arnold et al., 2011). This is supported by research by Stair et al (2023), which states that the INS-GAS animal model using PMSS1 can maintain CagA longer and promote H. pylori-related iron deficiency. Strain 7.13 is a CagA-positive strain with a functional cag type IV secretion system, and there is a mutation in vacA that abrogates VacA production (Beckett et al., 2016). Strain 7.13 is a gerbil derivative of H. pylori strain B128, which was originally isolated from a United States gastric ulcer patient infected with MG.

In research by Su et al (2022), it was discovered that strain 7.13 was able to cause gastric metaplasia and atrophy in MG for 2- 10 weeks. Another commonly used strain is ATCC 26695 which was isolated from gastric cancer patients. This strain has been well documented to cause mild to severe gastritis with atrophy (Chen et al., 2019; Mishra et al., 2019; Marquesz et al., 2018) accompanied by increased TNF-α (Chen et al., 2019), IL-33 (Kuo et al., 2019) and IgG (Chen et al., 2018; Mishra et al., 2019). Meanwhile, strain

ATCC 43504 is a strain that contains strong VacA and causes severe mucosal injury in short-term infections (Hong et al., 2018). Variations in H pylori strains are strongly associated with inflammatory responses and gastric disease. Two major virulent factors of H. pylori are reported to induce pathogenesis: Cytotoxin associated gene A (CagA) and vacuolating cytotoxin A (VacA). CagA, encoded by the cag-pathogenicity island (PAI), is delivered to host cells via the Type IV secretion system (T4SS) (Tegtmeyer et al., 2017). The presence of the cag pathogenicity island (PAI) in H. pylori is associated with increased mucosal inflammation and increased risk of developing gastric cancer or peptic ulcer disease (Feng et al., 2020). Disease severity is higher with CagA- positive strains compared with CagA- negative strains (Suzuki et al., 2019). The strains commonly used in research on H. pylori infection are SS1 and PMSS1. It is known that SS1 strains are commonly used to establish animal models of gastritis and intestinal metaplasia. Interestingly though both of these strains are CagA-positive and can efficiently infect animal models. However, it is known that PMSS1 can induce a more severe inflammatory response in infected mouse models (Peng et al., 2018). This is because SS1 undergoes recombination in Y cells (Amalia et al., 2022) which disrupts T4SS function and loses cag PAI function (Morningstar et al., 2022) thereby reducing its tendency to inflammation. The PMSS1 strain showed high virulence and pathogenicity (Ray et al., 2021; Tang et al., 2023), while the SS1 strain showed high colonization in the mouse stomach compared with other strains (Liu et al., 2020).

Currently, various tests are available to detect H. pylori. This test involves both non-invasive and invasive methods. Non- invasive examinations, such as urea breath test (UBT), H. pylori stool antigen test (HpSA), and serological tests. This method is considered faster, more economical and practical. However, it is considered less specific considering that H. pylori antibodies may remain positive for several months or more after eradication of the bacteria and it is known that comorbidities can affect sensitivity (Han et al., 2020). The non-invasive method more widely used in experimental animal models is the ELISA serological test to check IgG levels in screening for infection. It is known that H. pylori infection is characterized by an increase in IgG in the blood (Song et al., 2022; Hong et al., 2018).

On the other hand, invasive methods, such as histopathology, H. pylori culture, rapid urease test (RUT) or CLO and modern molecular tests such as PCR provide high sensitivity and specificity, however these methods can cause irritation and cause trauma because the examination requires a biopsy gastric mucosa (Farouk et al., 2018; Trung et al., 2019). Rapid Urease Test (RUT) and is also known as the Campylobacter-like organism (CLO). The RUT test is commonly used in experimental animal models which is characterized by a change in indicator color from yellow to pink or purple as a positive sign (Hong et al., 2018; Song et al., 2022; Li et al., 2023; Chen et al., 2019; Wei et al., 2019; Mishra et al., 2019). Histopathological examination to check for H. pylori infection is also often used using several stains such as hematoxylin and eosin (H&E), Giemsa, Warthin-Starry (WS), and modified methylene blue. On H&E (Sosa et al., 2020; Chen et al., 2018, Mishra et al., 2019) and gram staining (Wei et al., 2019; Ragab et al., 2022; Chen et al., 2018) H. pylori colonies pink or purple colored curved or rod shaped, while on Giemsa examination (Hong et al., 2020, Chen et al., 2022; Chen et al., 2018; Li et al., 2023, Marquez et al., 2018) and Methylene Blue (Tang et al., 2023) the colonies will be blue, and in the Warthin-Starry examination (Xue et al., 2020; Chen et al., 2018) the colonies colonies will be black or with a yellow background. Examination of H. pylori specific genes such as CagA, VacA, UreA, UreB can be done using the PCR system (Wei et al., 2019; Gonciarz et al., 2019; Shao et al., 2018; Saha et al., 2022).

4. CONCLUSIONS

In order to successfully create an experimental animal model of H. pylori, several important aspects must be taken into account, such as the animal model, H. pylori strain, number of inoculations, length of colonization and appropriate confirmation methods. C57BL/6, BALB/c and Mongolian gerbil animal models are commonly used in H. pylori research. C57BL/6 and BALB/c are capable of producing gastritis, metaplasia and hyperplasia and rarely produce cancer, while Mongolian gerbils are considered more susceptible to creating cancer models. The strains commonly used in H. pylori infection research are SS1 and PMSS1 because they are thought to be CagA-positive and can infect animal models efficiently. Specific animal models can also be created using transgenic animal models to analyze the effect of certain genetic composition or expression on H. pylori pathogenicity. Methods for detecting H. pylori are divided into 2, namely non- invasive and

invasive methods. The non- invasive method most often used in experimental animal models is the ELISA serological test to check IgG levels, while the invasive methods often used are RUT and H&E as well as additional staining such as gram stain, Giemsa, Methylene Blue and Warthin-Starry. The PCR method is also useful in examining specific H. pylori genes.

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