UNIVERSITI PUTRA MALAYSIA

SYSTEMATIC REVIEW ON PREVALENCE, CLINICAL CHARACTERISTICS AND DIAGNOSIS OF BRUCELLA CANIS IN DOGS IN ASIA FROM 2000 TO 2021

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SYSTEMATIC REVIEW ON PREVALENCE, CLINICAL CHARACTERISTICS AND DIAGNOSIS OF *BRUCELLA CANIS* IN DOGS IN ASIA FROM 2000 TO 2021

ARINAH NABIHAH BINTI AHMAD

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DEDICATION

This thesis is especially dedicated to:

My loving parents

Ahmad Bin Abdul Karim and Wan Norazlina Binti Wan Abdul Ghani

My supportive supervisors

Dr. Mazlina Mazlan

Assoc. Prof. Dr. Gayathri Thevi Selvarajah

And

Fellow DVM Friends

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First and foremost, praises and thanks to Allah, the Almighty, for his showers of blessings throughout my final year project to complete the project successfully.

I want to express my heartfelt gratitude to my loving parents for their love, prayers, caring and support through all of the ups and downs of completing this project.

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Also, I would also like to thank to my friends for their support and assistance throughout this project especially to Tharishine, Imran Ariff, Maisarah, Syifa, Afiqah and also to all my friends and classmates who constantly show me support and care not only in doing the final year project but most importantly the journey throughout the DVM program.

Lastly, I would like to thank my siblings and my seven cats for always cheering me up when I am sad and being the happy pills of my life.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir.

ULASAN SISTEMATIK PREVALENS, CIRI-CIRI KLINIKAL DAN DIAGNOSIS *BRUCELLA CANIS* PADA ANJING DI ASIA DARI TAHUN 2000 HINGGA 2021

Oleh

Arinah Nabihah Binti Ahmad

2021

Penyelia : Dr. Mazlina Mazlan

Penyelia bersama : Prof. Madya Dr. Gayathri Thevi Selvarajah

Bruselosis anjing ialah penyakit berjangkit zoonosis yang disebabkan oleh *Brucella canis*, sejenis kokobasilus gram-negatif dari keluarga Brucellaceae. Ia adalah penyakit yang telah dilaporkan di seluruh dunia dan boleh menyebabkan masalah kesihatan awam kerana hubungan rapat antara anjing dan manusia. Masih terdapat kekurangan kajian epidemiologi yang konsisten berkenaan prevalens bruselosis anjing di Asia. Kajian ini dijalankan dengan tujuan untuk mengkaji secara sistematik prevalens, ciri klinikal dan diagnosis jangkitan *Brucella canis* pada anjing daripada penerbitan yang melibatkan negara-negara Asia dari tahun 2000 dan 2021. Kajian ini dibuat menurut

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garis panduan 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA). Carian literatur telah dijalankan melibatkan tiga pangkalan data (PubMed: 218, Scopus: 449 dan Google scholar: 889) dengan tumpuan pada penerbitan dalam bahasa Inggeris dan berdasarkan kata kunci carian yang spesifik. Sebanyak 32 buah artikel jurnal memenuhi kriteria pemilihan dan layak untuk ulasan sistematik. Prevalens *Brucella canis* di negara Asia dilaporkan antara 0% dan 50%. Tiga ujian diagnostik yang biasa digunakan termasuk kultur dan pengenalpastian bakteria (n=11), ujian pengaglutinatan slaid pantas (RSAT) (n=10) dan reaksi rantai polimerase (PCR) (n=8). Antara ciri-ciri klinikal yang biasa dilaporkan ialah keguguran (n=9), limfadenopati (n=3) dan metritis (n=3). Bruselosis anjing adalah mencabar untuk didiagnosis dan bergantung pada beberapa aspek, termasuk penggunaan beberapa ujian diagnostik, pensampelan spesimen yang tepat pada selang masa tertentu dan pengenalpastian tanda klinikal yang berkaitan dengan bruselosis anjing yang kadang-kala bersifat tidak spesifik pada anjing. Walau bagaimanapun, masih terdapat batasan dalam kajian ini disebabkan oleh kekurangan kes yang dilaporkan di Asia dan kajian ini mungkin terlepas kes yang diterbitkan dalam jurnal tempatan atau jurnal bukan berbahasa Inggeris.

Kata Kunci: *Brucella canis*; anjing, kelaziman, diagnosis, ciri klinikal, Asia

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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999 - Final Year Project.

SYSTEMATIC REVIEW ON PREVALENCE, CLINICAL CHARACTERISTICS AND DIAGNOSIS OF *BRUCELLA CANIS* IN DOGS IN ASIA FROM 2000 TO 2021

By

Arinah Nabihah Binti Ahmad

2021

Supervisor : Dr. Mazlina Mazlan

Co-supervisor : Assoc. Prof. Dr. Gayathri Thevi Selvarajah

Canine brucellosis is a zoonotic infectious disease caused by *Brucella canis*, a gram-negative coccobacillus from the family Brucellaceae. It is a disease that has been reported worldwide and can cause public health problem due to close contact between dogs and humans. There is still a lack of consistent epidemiological studies on the prevalence of canine brucellosis in Asia. This study was conducted with the aim to systematically review the prevalence, clinical characteristics and diagnosis of *Brucella canis* infection in dogs from publications involving Asian countries from 2000 and 2021. This review was done in accordance to the Preferred Reporting Items for

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Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Literature search was conducted on three databases (PubMed: 218, Scopus: 449 and Google scholar: 889) with publications in English and based on specific search keywords. A total of 32 published articles met the selection criteria and were eligible for systematic review. The prevalence of *Brucella canis* in Asian countries was reported between 0% and 50%. The three commonly used diagnostic tests include bacterial culture and identification (n=11), rapid slide agglutination test (RSAT) (n=10) and polymerase chain reaction (PCR) (n=8). Among the common clinical presentations reported were abortion (n=9), lymphadenopathy (n=3) and metritis (n=3). Canine brucellosis can be challenging to diagnose and is dependent on several aspects, including the use of several diagnostic tests, accurate specimen sampling at specific time intervals, and identification of the clinical signs associated with canine brucellosis which sometimes can be non-specific in dogs. However, there is still limitation in this study due to lack of cases reported in Asia and this study may have missed the cases published in local or non-English-language journals.

Keywords: *Brucella canis*; dogs, prevalence, diagnosis, clinical characteristics, Asia

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1.0 Introduction

Canine brucellosis is a zoonotic infectious disease caused by *Brucella canis (B. canis)*, a gram-negative coccobacillus in the family Brucellaceae (class Alphaproteobacteria) (Spickler, 2018). This disease was first recognized in 1966 in the United States of America (Mosallanejad et al., 2009). *Brucella canis* appears to be widespread, with reports coming from North, Central, and South America, Africa, Europe as well as parts of Asia (Spickler, 2018). *Brucella canis* is an important cause of reproductive failure in dogs, especially in kennels. This is a contagious disease that spreads by venereal and oral routes. Infections in females are manifested as abortions and stillbirths, while in males, epididymitis, prostatitis, orchitis, and sperm abnormalities are commonly reported. However, some dogs remain asymptomatic and can become carriers. This disease is a potential zoonosis because canine brucellosis has been reported to infect humans (Khairani-Bejo et al., 2006). Direct contact with infected bitches' vaginal discharges and ingestion of contaminated placental materials or aborted foetuses can cause infection. Furthermore, infected males may also shed the organisms through urine (Mosallanejad et al., 2009). Despite the fact that several antibiotic therapies have been attempted, there is currently no known cure for this disease. Even after treatment, intact animals should be neutered, and treated dogs should be isolated. This is due to the fact that the therapy is not curative and carriers may still pose a risk to other dogs and humans (Makloski et al., 2011). It is still unclear on how important *B. canis* is as a source of human illness. This is because only a few clinical cases in people have been reported, and with most of them being mild cases. On the other hand, human cases may be underdiagnosed since the symptoms are

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generic and nonspecific, clinicians' diagnostic suspicion is low, and to get a definitive diagnosis is challenging (Spickler, 2018). There is a lack of consistent epidemiological studies on the prevalence and clinical characteristics of canine brucellosis in Asia, but occurrence of cases have been reported in some of the countries in different parts of Asia such as Malaysia, Turkey and India (Khairani-Bejo et al., 2006; Öncel et al., 2005; Sharma et al., 2012).

1.1 Justification

There is still a lack of consistent epidemiological studies on the prevalence and clinical characteristics of *Brucella canis* in Asia and no systematic review has been done regarding to this topic.

1.2 Research Objective

The objective of this study is to systematically review the prevalence, clinical characteristics and diagnosis of *Brucella canis* infection in dogs from publications involving Asian countries from 2000 and 2021.

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2.0 Materials and Methods

2.1 The review protocol

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

2.2 Formulation of research questions

The research question for this study was developed using PICo. PICo is a tool that assists authors to come up with appropriate research questions for their reviews. PICo is built on three main pillars, which are Population or Problem, Interest and Context. The authors have incorporated three general aspects namely dog (Population), prevalence, clinical characteristic and diagnosis of *Brucella canis* infection (Interest) and Asia (Context) based on the concept. This then helped guide the authors to develop the main research question- What are the prevalence, clinical characteristics and diagnosis of canine brucellosis in dogs caused by *Brucella canis* in Asia?

2.3 Search strategy

A total of three online scientific databases namely Scopus, PubMed and Google scholar were used to systematically search the relevant reports to conduct this study. Boolean operators were used to conduct the search strategy with the following search terms: ("canine brucellosis" OR "*Brucella canis*"). Only studies published in english between 2000 and 2021 were included in this study. Asia were not included in the search strategy because most of the title only put the name of specific country instead of Asia.

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2.4 Quality assessment

In this systematic review, only articles published between 2000 and 2021 were included. A total of 1556 studies were found in three databases during literature search (PubMed: 218, Scopus: 449 and Google scholar: 889). Duplicated articles were excluded from this study, of which 940 articles were then left for screening. Subsequently, using the following exclusion criteria, title and abstract screening were performed to choose suitable articles: (1) Studies involving the detection of human brucellosis and other types of animals rather than dogs. (2) Studies involving other types of brucella species and other diseases. (3) Studies involving other than Asia countries. (4) Experimental, molecular, genomic, sequencing and vaccine studies. (5) Review article, systematic review and meta-analysis, editorials, thesis conference paper and non-peer reviewed article (6) non-english articles. The citations and abstract of the articles were exported into the excel spreadsheets and Mendeley for managing purposes Then, 41 of the selected articles underwent a full text review for assessment of their eligibility for inclusion. Following the full-text review, 32 articles met the inclusion criteria and were eligible to be included in this systematic review (Figure 1).

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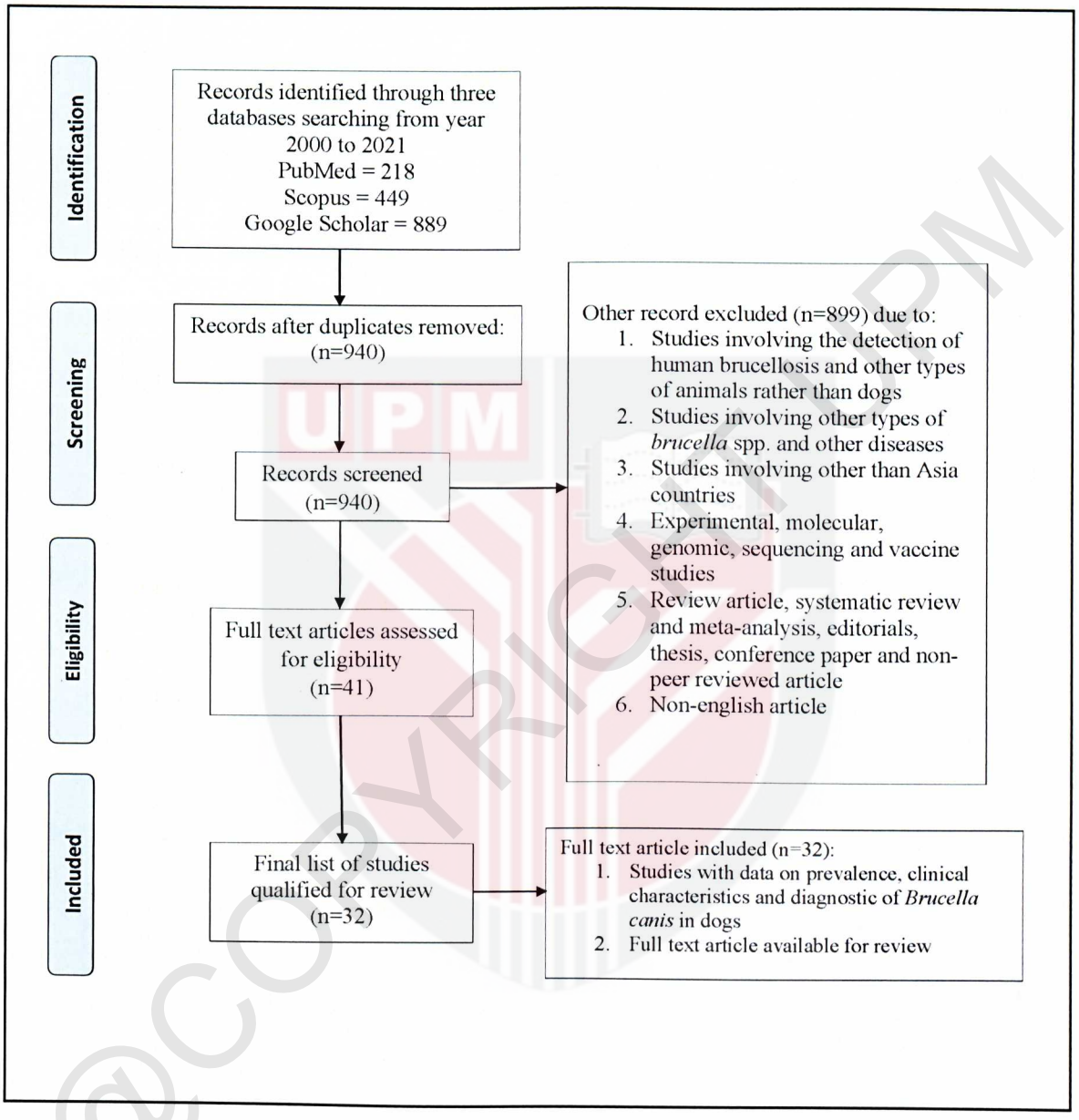

Figure 1: Flow diagram of articles selection process applied in the systematic review

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2.5 Data extraction and analyses

Following eligibility, the data from eligible studies were extracted and documented in Microsoft Office Excel and Microsoft Words as follows: type of samples (blood, serum, tissues, vaginal and preputial swabs), the total number of samples tested and the number of positives and types of diagnostic tests such as serological test, (tube agglutination test [TAT], microplate agglutination test [MAT], rose bengal plate test [RBPT], rapid slide agglutination test [RSAT], latex agglutination test, immunofluorescence assay [IFA], Dot-ELISA, Indirect-ELISA, immunochromatography assay [ICA] and agar gel immunodiffusion assay [AGID]), bacterial culture and identification and molecular test (polymerase chain reaction [PCR]) and other tests (histopathology and immunohistochemistry [IHC]). In this review, descriptive analyses of extracted data were performed, which were presented together with a narrative synthesis. In addition, the map was created using online website named MapChart (https://mapchart.net/asia.html).

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3.0 Results

3.1 Study Characteristic

Figure 2: Geographical distribution of articles (n=32) included in the review

The review managed to obtain 32 selected articles. Since this study only selected articles from Asia, a total of 13 countries had reported cases of *Brucella canis* infection in dogs. Figure 2 represents the geographical distribution of studies that had reported *Brucella canis* cases in dogs in Asia which included countries like Japan, Korea, India, Turkey, Iran, China, Pakistan, Thailand, Bangladesh, Malaysia, Iraq, Israel and Jordan. Out of the 32 articles, Turkey (n=9) recorded the highest number of articles on *Brucella canis* in dogs, followed by Korea (n=6), Japan (n=3), India (n=2) and

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China (n=2), while Pakistan, Thailand, Bangladesh, Iraq, Malaysia, Israel and Jordan recorded the lowest number of articles (n=1). Figure 3 represents the number of articles reported in Asia.

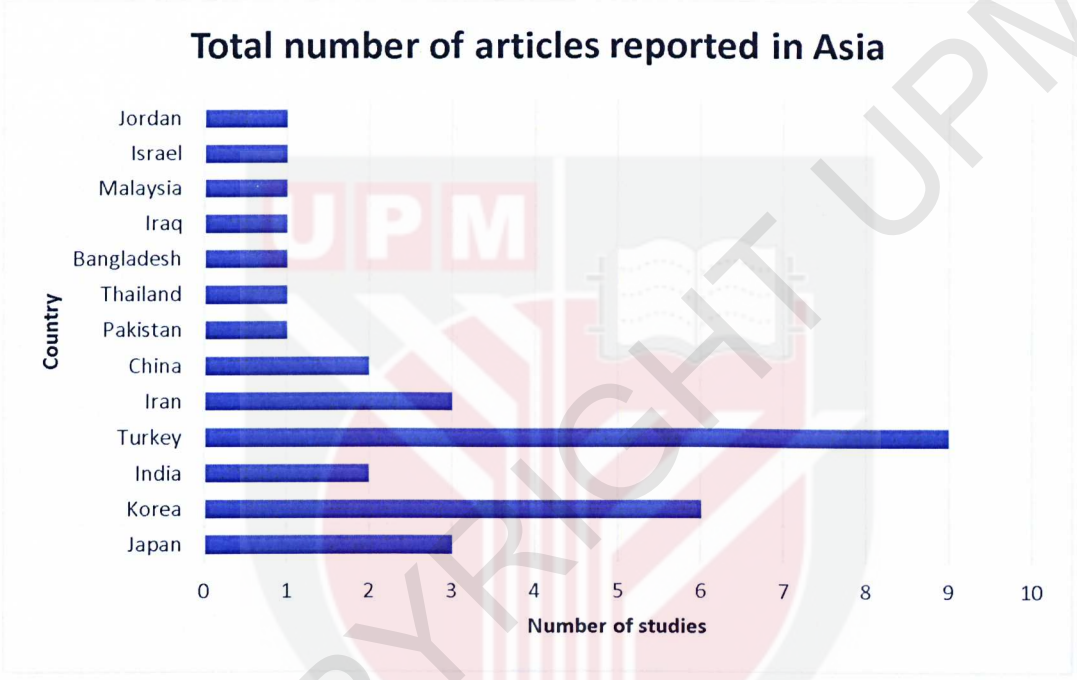

Figure 3: Number of articles reporting clinical signs of *Brucella canis* infection in dogs in Asia from 2000 to 2021

There were three common diagnostic methods used to detect *Brucella canis* in dogs which included serological, bacteriological and molecular detection. However, there were other tests that can be conducted such as histopathology and immunohistochemistry to detect *Brucella canis* infection in dogs.

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3.2 Prevalence and diagnosis of *Brucella canis* infections in dogs in Asia

3.2.1 Serological detection

There were 26 eligible articles that reported the prevalence and seroprevalence of *Brucella canis* in Asia. From these articles, several serological methods were performed to detect *Brucella canis* which included tube agglutination test, rapid slide agglutination test, rose Bengal plate test, immunochromatography assay, indirect Enzyme-linked immunosorbent assay (i-ELISA), Dot Enzyme-linked immunosorbent assay (Dot-ELISA) immunofluorescence assay and agar gel immunodiffusion assay (Table 1). The prevalence of *Brucella canis* infection in dogs in Asia from 2000 to 2021 varies between 0% and 50% in which the variation depends on the test method conducted.

Table 1: Total seropositive dogs according to the serological method

Diagnostic Test	No. of articles	Total number of animal positive/Total number of animal-tested (n)	References
TAT	6	n=147/2849 (5.2%)	(Öncel et al., 2005) (Watarai et al., 2007) (Tsogtbaatar et al., 2008) (Sharma et al., 2012) (Castillo et al., 2014) (Saytekİn et al., 2018)
2ME-TAT	5	n=78/1149 (6.8%)	(Öncel et al., 2005) (Khairani-Bejo et al.,2006) (Zeki Aras et al., 2010) (Sharma et al., 2012) (Saytekİn et al., 2018)

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MAT/SAT/MPAT	6	n=135/2402 (5.6%)	(Kimura et al., 2008) (Zeki Aras et al., 2010) (Castillo et al., 2014) (Bastan & Bas, 2017) (Ergene et al., 2018) (Jamil et al., 2019)
RSAT	4	n=76/274 (27.7%)	(Zeki Aras et al., 2010) (Zeki Aras, 2010) (Saytekİn et al., 2018) (Zhong et al., 2020)
2ME-RSAT	6	n=270/1364 (19.8%)	(Kim et al., 2007) (Bae, 2009) (Mitra et al., 2013) (Alshehabat & Obaidat, 2019) (Bardenstein et al., 2021) (Erbas et al., 2020)
RBPT	6	n=126/1779 (7.1%)	(Khairani-Bejo et al., 2006) (Sharma et al., 2012) (Xiang et al., 2013) (Rahman et al., 2015) (Zhong et al., 2020) (Bardenstein et al., 2021)
Latex agglutination test	1	n=12/318 (3.8%)	(Watarai et al., 2007)
ICA	8	n=293/4369 (6.7%)	(Kim et al., 2007) (Bae, 2009) (Hong et al., 2010) (Behzadi & Mogheiseh, 2011) (Mitra et al., 2013) (Mosallanejad et al., 2013) (Jung et al., 2018) (Hams et al., 2016)
i-ELISA	7	n=165/1102 (15%)	(Öncel et al., 2005) (Tsogtbaatar et al., 2008) (Zeki Aras et al., 2010) (Zeki Aras, 2010) (Rahman et al., 2015)

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			(Hams et al., 2016) (Yumusak et al., 2017)
Dot-ELISA	4	n=53/699 (7.6%)	(Zeki Aras, 2010) (Sharma et al., 2012) (Ngamkala et al., 2020) (Bardenstein et al., 2021)
IFA	1	n=15/95 (15.8%)	(Akhtardanesh et al., 2011)
AGID	2	n=11/548 (2%)	(Sharma et al., 2012) (Bardenstein et al., 2021)

From the systematic review, it was found that the agglutination test for antibody detection in serum sample from dogs ranges from 0% to 50%. The seropositivity of the *Brucella canis* infection in dogs in Asia by using agglutination test is 8.1% (refer to Appendix 1). However, there are several types of agglutination test such as TAT, MAT, RSAT, RBPT and latex agglutination test.

From the serological detection, rapid slide agglutination test was found to be the most commonly used serological method in this study with a total of ten articles. Rapid slide agglutination test (RSAT) reported the highest seroprevalence in India at 50% (Mitra et al., 2013) and the lowest seroprevalence was reported in Turkey with 6% (Erbas et al., 2020). The second highest serological detection conducted in Asia was TAT in which the highest seroprevalence was reported in Turkey with the seroprevalence of 22.2% (Zeki Aras et al., 2010) and the lowest prevalence reported in Japan at 1.2% (Castillo et al., 2014). There were a few papers that incorporated the use of 2-mercaptoethanol into the agglutination test such as the 2-mercaptoethanol tube agglutination test (2ME-TAT) and 2-mercaptoethanol rapid slide agglutination test (2ME-RSAT). Active and inactive infection can be distinguished by using 2-

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mercaptoethanol agglutination tube (Khairani-Bejo et al., 2006). In Malaysia, there was only 1 published article on *Brucella canis* in dogs which was detected by using 2ME-TAT with a prevalence of 4.8% (Khairani-Bejo et al., 2006). There was a published article in Turkey that conducted both TAT and 2ME-TAT. The seroprevalence of using TAT was 12.7% which was higher than the seroprevalence of *Brucella canis* conducted using 2ME-TAT reported at 7.73% (2ME-TAT) (Öncel et al., 2005).There were also six studies using the microplate agglutination test (MAT) to detect *Brucella canis* infection in dogs. The studies conducted using the MAT reported the highest seroprevalence in Pakistan (37.6%) (Jamil et al., 2019) and lowest seroprevalence in Turkey (0.8%)(Bastan & Bas, 2017). The seropositive of *Brucella canis* infection in dogs by using microplate agglutination test is 5.6%.

There were six published articles reported detection of *Brucella canis* by using RBPT with seropositivity of 6.55%. The highest reported seroprevalence using RBPT was in India with seroprevalence of 11.76% (Sharma et al., 2012) and the lowest seroprevalence was reported in Malaysia with the 0% seroprevalence. This is due to all of the serum samples in Malaysia were tested negative of *Brucella canis* by using RBPT (Khairani-Bejo et al., 2006). However, most of the studies used RBPT antigen prepared from *Brucella abortus* (Strain 99) and some studies used specific *Brucella canis* antigen such as *Brucella canis* strain MEX-511 as well as rough *Brucella canis* LPS antigens. Lastly, only one study conducted the latex beads agglutination test which reported seroprevalence of 3.77% in Japan (Watarai et al., 2007). From the several agglutination tests reported, RSAT and TAT were reported with the most number of studies (n=11 and 10) in Asia.

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Next, the other serological method that reported with highest number of studies (n=8) is ICA. The seropositives of *Brucella canis* in dogs detected using ICA in Asia was 6.71% with highest seroprevalence reported in India (50%) (Mitra et al., 2013) and lowest seroprevalence reported in Korea (2.5%) (Hong et al., 2010). Besides that, there were four studies in Korea that conducted ICA to detect the *Brucella canis* infection in dogs. This showed that this test was widely used in Korea and it reported highest seroprevalence at 39.1% in 2007 (Kim et al., 2007). However, in 2018, the reported seroprevalence become much lower at 2.5% (Hong et al., 2010). Other serological tests that were also observed in our systematic review included agar AGID which was only conducted in two studies with seropositive results of 2% for *Brucella canis*. The only seroprevalence reported was in India which reported seroprevalence at 1.5% (Sharma et al., 2012). Next, IFA was reported only in Iran with prevalence of 15.8% (Akhtardanesh et al., 2011).

Enzyme-linked immunosorbent assay (ELISA) is also one of the serological methods that can be used to detect the prevalence of *Brucella canis* infection in dogs. From this review, there were two types of ELISA used to detect *Brucella canis* infection in dogs such as indirect enzyme-linked immunosorbent assay (i-ELISA) and dot enzyme-linked immunosorbent assay (dot-ELISA). This review revealed a total of seven studies conducted i-ELISA and four studies performed dot-ELISA. For i-ELISA, there was one study which used recombinant SOD coated on the Immuno plates (Tsogtbaatar et al., 2008) and the rest of the studies were performed and standardized according to the method by Mateu-de-Antonio et al. (1993). The seropositivity of *Brucella canis* using i-ELISA was 14.97%. The highest

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seroprevalence of *Brucella canis* infection in dogs detected by using i-ELISA was in Turkey at 24.4% (Zeki Aras et al., 2010) while the lowest seroprevalence was reported in Bangladesh with 4% (Rahman et al., 2015). In addition, some of the studies used only selective samples from positive dogs as a confirmatory test and thus, could not be regarded as seroprevalence. Next, for dot-ELISA, there were two studies that detected *Brucella canis* antibodies performed by using the ImmunoComb Canine Brucella Antibody Test Kit® (Biogal-Galed Laboratories Israel) and the other two studies conducted using dipstick enzyme immunosorbent assay. The seropositivity of *Brucella canis* infection in dogs when detected with dot-ELISA, was 7.58%. The highest seroprevalence when conducted with dot-ELISA was reported in Turkey (23.7%) (Zeki Aras, 2010) and the lowest prevalence was reported in India (3.03%) (Sharma et al., 2012).

Therefore, the seroprevalence of *Brucella canis* varies between 0% and 50% in serological studies on dogs conducted in different countries in Asia. The lowest seroprevalence of *Brucella canis* was reported in Malaysia using RBPT whilst the highest seroprevalence was reported in India using 2ME-RSAT and ICA.

3.2.2 Bacterial culture and identification

Bacterial culture and isolation reported the highest number of studies in this systematic review with a total of 11 articles. The countries that performed this diagnostic test are Korea, Turkey, China, Malaysia and Israel. The type of samples collected from the dogs were blood, tissues, urine and swab samples from the reproductive organ. Tissues that were obtained for bacterial isolation included genital organs such as uterus, testis,

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epididymis and prostate glands, mammary glands, lymph nodes (superficial inguinal and retropharyngeal), liver and kidney (Jung et al., 2020). However, the most common sample collected in these studies was blood which was reported by nine articles. The overall seropositive of *Brucella canis* detected from the blood sample was 13.3%. The prevalence of *Brucella canis* detected by bacteriological detection varies from 0% to 24.8%. The highest prevalence was reported in Korea at 24.8% and the lowest prevalence reported in Malaysia at 0% as there was no isolation of *Brucella canis* from the study (Khairani-Bejo et al., 2006). Next, there were two studies which used tissues collected from dogs that had died because of unknown causes (Jung et al., 2020) and dogs euthanized due to being positive for *Brucella* sp. during the screening test (Aras & Uçan, 2010). The inguinal lymph nodes were procured in the study by Aras and Uçan (2010) and *Brucella* was isolated from four of 48 samples. Next, there were many types of tissue samples collected from the study done by Jung et al. (2020). For instance, they collected lymph nodes (superficial inguinal and retropharyngeal lymph nodes), liver, kidney, mammary gland, uterus, testis, epididymis and prostate. However, the samples the with the highest bacterial isolation rate was the superficial inguinal lymph nodes (77.5%). There were no isolation of *Brucella canis* from the study in Malaysia (Khairani-Bejo et al., 2006) and Turkey (Yumusak et al., 2017). From the urine sample collected in a study in Korea, 24 out of 35 were found to be positive (Jung et al., 2020). Other than that, bacterial isolation from swab samples of reproductive tract was attempted in a few countries such as in Turkey and Israel. There was no successful isolation from the preputial swab sample of the male study done by Yumusak (2017), while the vaginal swab samples managed to isolate *Brucella canis*.

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This is partly because the female dogs had history of abortion (SaytekİN et al.,2018; Bardenstein et al., 2021).

3.2.3 Molecular detection: Polymerase Chain Reaction

A total of 8 articles in Asia reported performing Polymerase chain reaction (PCR) for detection of canine brucellosis. The studies were reported in Japan, Turkey, China, Iraq and Israel. The prevalence of *Brucella canis* detected by PCR varies from 1.8% to 8.3%. There are different types of samples used in the published articles such as blood and tissue samples as well as vaginal and preputial swabs. However, studies that presented PCR results were reviewed critically and were found that the amplification sites of DNA were different as shown in the Table 2. Screening of blood using PCR attempted in Turkey revealed positivity rate of 5% (Erbas et al., 2020). The screening test in this study was performed using *B. canis*-specific PCR procedures which were carried out according to Kang et al. (2014). Other than that, screening of tissue samples of dead dogs using PCR also attempted in Turkey revealed 8.33% positivity rate (Aras and Uçan, 2010). In this study, tissue samples that were used for screening were lymph nodes from the dogs that had died due to unknown causes. In 2017, there was a published article in Turkey that conducted PCR by taking the reproductive organ swab sample of dogs with positivity rate of 1.8% (Yumusak et al., 2017). Moreover, there were also some published articles performed PCR by using samples from positive tested dogs as a confirmatory test such as studies in Japan (Watarai et al., 2007) and Iraq (Hams et al., 2016). In Japan, the blood samples were taken from the positive dogs detected using the tube agglutination test and the positivity rate was 47.1% where only five out of 12 dogs were detected positive by PCR (Watarai et al., 2007).

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Moreover, in Iraq, the positivity rate reported using PCR was 16.13% from the seropositive samples (Hams et al., 2016). Turkey, China and Israel were countries that reported with several case reports for detection of *Brucella canis* in dogs by using PCR. All the cases reported 100% positivity rate in several samples such as blood and vaginal swab. In Turkey, *Brucella canis* was isolated from vaginal swab and blood samples of two dogs which had previously aborted (Saytekİn et al., 2018). Zhong et al., (2020) reported that the two isolates (Y4 and W5) from the bacterial culture and identification produced a unique 300-bp amplicon in BcSS-PCR tests, indicating that they were *Brucella canis*. In 2019, *Brucella canis* was also detected in five out of five blood samples from dogs and this was the first reported outbreaks of *Brucella canis* in Israel (Bardenstein et al., 2021).

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Table 2: Primers used for the amplification of the target gene

Target Gene	Primers	Sequence (5' → 3')	Product	References
Multiplex PCR	B2F B2R	TCATGAAAACCGCTTCCCCCAGC CCGTTGTCATGATCTGTGTCCCT	332 bps	(Watarai et al., 2007)
BCSS	BCAN B0548 BCAN B0549	CCAGATAGACCTCTCTGGA TGGCCTTTTCTGATCTGTTCTT	300 bps	(Zhong et al., 2020) (Erbas et al., 2020)
Polysaccharide deacetylase	BMEI1436f BMEI1435r	ACG CAG ACG ACC TTC GGT AT TTT ATC CAT CGC CCT GTC AC	794 bps	(Saytekİn et al., 2018)
BCSP31	B4 B5	TGGCTCGGTTGCCAATATCAA CGCGCTTGCCTT-TCA-GGT-CTG	224 bps	(Zhong et al., 2020) (Saytekİn et al., 2018)
16S-23S rDNA ITS region	ITS66 ITS279	ACATAGATGCCAGGCCAGTCA AGATACGGACCGAACGCTAC	214 bps	(Aras & Uçan, 2010) (Bardenstein et al., 2021) (Yumusak et al., 2017)

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3.2.4 Histopathology and immunohistochemistry

There were two articles that reported histopathological findings of *Brucella canis* infection in dogs. Both of the studies used tissue samples such as lymph node, liver, spleen, lung and kidney. The highest prevalence of microscopic lesion is mild to severe lymphohistiocytic interstitial inflammation of prostate gland which was presented in 81.8% of dogs in Korea (Jung et al., 2020). The liver was shown to have the most common microscopic lesion in non-reproductive organs which reported multifocal neutrophilic or lymphocytic hepatitis was observed in 75.0% dogs in Korea (Jung et al., 2020). In 34.5% (10/29) of female dogs, the mammary gland had multifocal interstitial lymphocytic infiltration (Jung et al., 2020). Moreover, lymph node swelling was seen in some dogs, and histopathologic lesion of lymphoid follicular hyperplasia was found in 47.5% (19/40) of the dogs (Jung et al., 2020). Diffuse lymphoreticular hyperplasia causes swelling of lymph nodes (Jung et al., 2020). In China, the histopathological findings observed in the dogs were extensive inflammatory and necrotic lesions in the liver, spleen, testicle, lymph node, lung, and kidney. Furthermore, the histopathological lesions observed in the liver were severe infiltration of lymphocytes and neutrophils detected in portal tracts. Other than that, microscopic lesions of severe necrosis of spermatogenic cells and damaged seminiferous tubule were observed in testis of the male dogs with significant infiltration of neutrophil, lymphocyte, and macrophage accumulation in interstitial tissues. However, the study in China only observed cases of male dogs and no female dogs were reported in this published article. Moreover, this study conducted in China also performed immunohistochemistry in tissues to detect *Brucella canis* infection in

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dogs. In portal infiltrates of the liver, *B. canis* antigens were mostly found in the cytoplasm of macrophages and neutrophils (Zhong et al., 2020). *Brucella canis* was also detected in cytoplasm of the macrophages, neutrophils and spermatogenic cells of testicle. The study of Zhong et al., (2020) reported proliferation of lymphocytes and reticuloendothelial cells, as well as deposition of fibrinous material identified in lymph nodes.

3.3 Clinical characteristics of *Brucella canis* infection in dogs

Table 2 shows the number of studies which reported clinical characteristics of *Brucella canis* infections in dogs. Out of the 32 published articles, there were 11 published articles which reported clinical characteristics notable in dogs infected with *Brucella canis*. The most common clinical presentation reported was abortion with a total of nine published articles. The second highest clinical presentation reported were lymphadenopathy and metritis in female dogs with a total of three studies reported. Asian countries that reported cases of abortion were India, Turkey, Korea, Jordan, China, Iran and Israel. In Jordan, 7.1% of breeding dogs reported with cases of abortion prior to blood collection (Alshehabat and Obaidat (2019). Turkey also reported a complaint of abortion in two bitches from breeding kennels (SaytekİN et al., 2018). Seven out of 21 seropositive dogs in Beijing, China had abortion as well as other reproductive abnormalities such as pyometra, epididymitis, scrotitis and metritis (Xiang et al., 2013). In Iran, there were three females from seropositive dogs which had abortion or stillbirth in their past history which can be observed in multiparous kennelled dogs (Akhtardanesh et al., 2011). Moreover, generalized lymphadenomegaly also were observed in this study. Bardenstein et al., (2021) also

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reported cases of abortion in the first reported case of *Brucella canis* outbreak in Israel. Zeki Aras et al. (2010) reported nine dogs from city pound of Konya, Turkey showed variable clinical signs such as lethargy, fever, anorexia as well as lymphadenopathy which were observed in the two female dogs. There were also studies of clinical characteristics reported from dead kennelled dogs that had history of repeated abortion and decreased fertility. The kennelled dogs were euthanized after being detected positive with *Brucella canis* (Jung et al., 2020). Abnormalities of musculoskeletal system also can be observed in the published articles by Akhtardanesh et al., (2011) and Mitra et al., (2013). In India, ten labrador bitches from the kennel with history of repeated breeding, abortion and some developed discospondylitis (Mitra et al., 2013). Report of discospondylitis in the study by Akhtardanesh et al., (2011) was observed only in a male dog that also showed rear limb paresis. Two out of 16 seropositive dogs in Iran reported ophthalmologic abnormalities of mild conjunctival hyperaemia, anterior uveitis and iris hyperpigmentation without the signs of systemic abnormalities (Mosallanejad et al., 2013).

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Table 3: Number of articles reporting clinical signs of *Brucella canis* infection in dogs in Asia from 2000 to 2021

Clinical characteristic	Number of articles	Clinical characteristic	Number of articles
Lethargy	2	Vaginal discharge	2
Fever	2	Pyometra	1
Decreased appetite	1	Unilateral testis	1
Anorexia	1	Balanoposthitis	2
Stillbirth	2	Enlarge testicle	1
Infertility	1	Metritis	3
Abortion	8	Discospondylitis	2
Lymphadenopathy	3	Osteomyelitis	1
Epididymitis	2	Uveitis	2
Scrotum dermatitis	2	Mild conjunctival hyperaemia	1
Orchitis	1	Iris hyperpigmentation	1
Testicular atrophy	1	Dermatitis	1

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4.0 Discussions

Studies on *Brucella canis* in Asia are limited and only 32 published articles met the inclusion criteria during the present study which illustrated the paucity of research on *Brucella canis* infection in dogs in Asia compared to the other parts of the world. Bacteriological, serological, and molecular testings are commonly used to diagnose brucellosis. From this study, the reported cases are mostly from shelter dogs, kennel dogs and stray dogs. This is because of the environmental factors where the dogs share the same environment. Crowded places such as by confining a large number of dogs in small-enclosed spaces or cages can be a perfect medium for the *Brucella canis* to spread among the dogs (Uçan, 2016). Next, the close contact with stray dogs and infected dogs also can be one of the factors contributing to the spreading of canine brucellosis. Erbas et al. (2020) found that the two cities in Turkey which are Aydın and Muğla have higher levels of positivity due to owned dogs were frequently in contact with stray dogs in the gardens of summer cottages or on the streets. In addition, due to high population of stray dogs, uncontrolled mating can also happen because most of the stray dogs are still intact. Other than that, any secretions and excretions produced by stray dogs living in the free environment are physiologically released into the environment which can easily spread to other dogs or human. Next, the lack of awareness among people about how important this disease also can be one of the factors. Bardenstein et al. (2021) reported that this disease is frequently overlooked as a cause of reproductive failure due to lack of expertise and experience, as well as a lack of awareness among veterinarians, dog owners, and breeders.

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Serological method remains as the most widely used diagnostic procedure (Alton et al 1988) as it provides fast and easy way to determine the infection's seroprevalence (Alton et al 1988; Öncel et al 2005). The seroprevalence of *Brucella canis* detected by serological method varies from 0% to 50%. India reported the highest seroprevalence at 50% by using ICA and 2ME-RSAT. This is because in this study, they collected the samples from kennel dogs and most of the dogs showed symptoms of canine brucellosis. However, in Malaysia, the seroprevalence of *Brucella canis* detected reported was 0% seroprevalence because of the diagnostic test used which is RBPT incorporated nonspecific *Brucella sp* antigen (Khairani-Bejo et al., 2006). So, this technique most likely could not detect the specific antibodies of the *Brucella canis*, thus the result is zero. However, Malaysia also conducted other test such as 2ME-TAT to get a better diagnosis. Interestingly, the seroprevalence of *Brucella canis* detected by 2ME-TAT was 4.8%.

From this present study, there are a variety of agglutination tests used to detect *Brucella canis* infection in dogs in Asia. Although agglutination tests reportedly have high sensitivity, their lack of specificity and the possibility of false positives warrants the use of a particular specific test (Kim et al., 2007). Furthermore, some of the studies also conducted using the additional of 2-mercaptoethanol in the agglutination test to lower the false positive result (Öncel et al., 2005). Positive TAT results were reduced by 40% with 2ME-TAT in the study of Öncel et al. (2005), and these findings were confirmed by ELISA. Cross-reactions with other pathogens such as the presence of *Pseudomonas aeruginosa*, mucoid *Staphylococcus* spp. and *Bordetella bronchiseptica*-specific antibodies also can result in false positive reaction (Erbas et

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al., 2020). Therefore, tube agglutination test should be paired with at least one other test, such as 2ME-TAT or ELISA, in order to eliminate false-positive results obtained in serological diagnosis of *B. canis* infection in dogs (Öncel et al., 2005). Furthermore, according to Jung et al. (2020), 2-mercaptoethanol (2-ME) was added to the assays to improve specificity by dissociating IgM, which can cause cross-reaction with other bacteria than IgG. However, sensitivity can also be reduced especially during the early stages of the immune response, when IgM predominates. Next, a study in Japan by Watarai et al. (2007) suggested the use of latex bead agglutination test as the test is simple to use, requires little to no technical knowledge and does not really require any special equipment. In addition, the test can be performed in the field and the result can be obtained within 15 minutes. However, more research is needed before it can be validated as a canine brucellosis diagnostic tool.

From the study by Jamil et al. (2019), the detection of *Brucella canis* is higher by using MAT but was found negative by PCR which can be due to persistent infection. After parturition or abortion, the infected bitches can shed huge amount of *Brucella canis*, however serological method do not produce accurate results during the first 12 weeks post-infections as well as the seropositivity largely dependent on bacteraemia (Mol et al., 2020). Some molecular techniques such as *B. canis* species specific PCR developed by Kang et al. (2010) also was used in two studies and claimed to be highly sensitivity. However, because of the lack of bacteria in circulation and localization in organs, a PCR test can yield negative results in cases of chronic infection. In addition, serological test might yield positive result due to antibodies were previously produced (Erbas et al., 2020). From the study of Aras and Uçan,

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(2010), *Brucella canis* was detected with PCR assay by using ITS66 and ITS279 pair of primers and the diagnostic sensitivity and specificity reported was 100%. This technique has the benefit of detecting *Brucella canis* directly from lymph node samples in just a day. It can also be used to confirm cultures and has a good specificity (Aras and Uçan, 2010).

This review also revealed that ICA is also one of the commonly performed test to detect canine brucellosis. From this study, India reported the highest seroprevalence detected using this method. This is because ICA is one of the method that is handy and can be regardred as a precise tool for rapid diagnosis of canine brucellosis (Mitra et al., 2013). Wanke et al. (2012) recommended that this test could be used for the routine clinical practice because the test is very simple to perform by following the manufacturer's instructions. Furthermore, the samples taken were from the kennel dogs with clinical characteristics of repeated breeding, abortion and discospondylitis. In 2007, a study in Korea reported one of the highest seroprevalence at 39.1% detected due to higher seropositivity of stray dogs. This is may be due to living conditions of the stray dogs, inadequate veterinary care and as well as uncontrolled mating (Jung et al., 2018).

Blood culture and isolation is the gold standard to detect *Brucella canis* infection in dogs. Additionally, blood cultures are essential for diagnosis, especially if serological results are ambiguous. However, the procedure for isolation of causative agent can be time consuming and the sensitivity may be affected by the intermittent absence of bacteraemia (Khairani-Bejo et al., 2006). Korea reported the highest prevalence (24.8%) because the collected samples were from 10 different breed

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kennels and canine brucellosis outbreak in Korea usually occurred in kennel dogs (Kim et al., 2007). On the other hand, Malaysia reported that no target organism was isolated from the blood samples. This could be related to the fact that *Brucella canis* takes a long time to culture, and its sensitivity could be affected by the intermittent absence of bacteraemia (Khairani-Bejo et al., 2006). Other factors that might interfere with successful isolation include antimicrobial therapy already given to the sampled dogs, the use of EDTA tube also can inhibit the growth of the bacteria as well as improper condition for storage and transportation of the samples (Santos et al., 2021). Thus, a negative culture should not rule out infection and it is recommended to perform additional diagnostic tests. From the study in Turkey, there was also unsuccessful isolation of *Brucella canis* from vaginal and preputial swab samples which may be due to the intermittent vaginal shedding of the agent as well as lower level of the bacteria during the time when the cases were conducted (Yumusak et al., 2017). This also can be due to some isolates may not grow well on certain media, hence using more than one medium is often recommended. Besides that, isolation of *B. canis* is not always successful especially in dogs chronically infected for an extended time (Spickler, 2018).

In molecular detection, the best source of sample for detecting asymptomatic or chronic *B. canis* infection were samples of the lymph nodes (Aras & Uçan, 2010). The lymph nodes, particularly those draining the retropharyngeal and inguinal areas are the best source of organisms in which the organism is likely to be discovered viable. Next, blood is the sample of choice due to prolonged bacteraemia of *Brucella canis* and the use of reproductive swab samples can detect the non-viable antigen (Aras

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& Uçan, 2010). Type of dogs selected for sample also can affect the prevalence of *Brucella canis* infection because the sample dogs used in Turkey varies from shelter, owned and stray dogs. Erbas et al., (2020) reported that PCR test can yield negative findings in cases of chronic infection due to the lack of bacteria in circulation and localization in organs such as the mesenteric lymph nodes. Serology may be positive in such circumstances since antibodies were previously produced. From the study conducted using histopathology, most internal organs of infected dogs showed lymphocytic or neutrophilic inflammation. On the other hand, the testis, epididymis, prostate gland, mammary gland, and uterus all showed multifocal to diffuse lymphocytic inflammation (Jung et al., 2020). In addition, *B. canis* antigen distribution in tissues and cell tropism can be determined using immunohistochemistry. According to Zhong et al., (2020), immunolabeling of *B. canis* antigens was found to be stronger in the spleen, testis and liver than in the kidney and lymph nodes, and this was linked to the severity of inflammatory and necrotic lesions in those tissues.

Bacterial culture and isolation followed by PCR can be useful for definitive diagnosis, but culture is not commonly available since it is time-consuming and needs a laboratory with suitable biosafety settings (Mol et al., 2020). Therefore, serological tests are more commonly used in diagnosis of *Brucella canis* since they are simple, practical, and provide quick results (Bastan & Bas, 2017).

In addition, *Brucella canis* in dogs typically causes reproductive problems which most commonly occur in sexually intact adult dogs. In female dogs, abortion and metritis can be seen due to infection of the uterus. Abortion which happens in the late stage of pregnancy usually occurs around days 45-55 of gestation resulting in birth

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to stillborn or the birth of weak puppies (Spickler, 2018). Other source of infection can also be attributed from surviving puppies that carry the bacteria and infect the people who are in close contact with them (Santos et al., 2021). They may also play a role in the pathogen's persistence in the canine community (Santos et al., 2021). Apart from that, dogs presented with lymphadenitis commonly involve retropharyngeal and inguinal lymph nodes as well as generalized lymphadenitis (Aras & Uçan, 2010). In male dogs, the most common clinical characteristic is epididymitis which can occur either in the acute or chronic phase. The epididymis increases in size during the acute stage and is often accompanied by pain and the presence of serosanguinous fluid in the tunica. Moreover, scrotal dermatitis and scrotal oedema also can result from frequent licking of the scrotum. In the chronic phase, the epididymis shrinks and hardens, and the testes frequently undergo atrophy (Wanke, 2004). However, asymptomatic infections also common in canine brucellosis (Aras & Uçan, 2010).

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5.0 Conclusion and Recommendations

Canine brucellosis can be challenging to diagnose and is dependent on several aspects, including the use of several diagnostic tests, accurate specimen sampling at specific time intervals, and identification of the clinical signs associated with canine brucellosis which can sometimes be non-specific in dogs. Most common clinical presentations reported are abortion, lymphadenopathy and metritis. However, presentation of a variety of clinical characteristics as well as asymptomatic infections also can happen. Nevertheless, there are still limitation in this study due to lack of cases reported in Asia and this study may have possibly missed the cases published in local or non-english-language journals. In addition, some countries may not subscribe to the databases that were used for this study that may be one of the limitation for this study. Thus, more studies of *Brucella canis* in Asia is warranted and the inclusion of other international databases can be done to get more articles or cases for future studies.

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References

Alshehabat, M., Obaidat, M., & Hayajneh, W. (2019). Seroprevalence of *Brucella canis* in dogs and at-risk humans in Jordan. *Veterinarni Medicina*, *64*(6), 260–265. https://doi.org/10.17221/67/2018-VETMED

Alton, G. G., Jones, L. M., Angus, R. D., & Verger, J. M. (1988). *Techniques for the brucellosis laboratory*. Institut National de la recherche Agronomique (INRA).

Aras, Z., & Uçan, U. S. (2010). Detection of *Brucella canis* from inguinal lymph nodes of naturally infected dogs by PCR. *Theriogenology*, *74*(4), 658–662. https://doi.org/10.1016/j.theriogenology.2010.03.023

Akhtardanesh, B., Ghanbarpour, R., Babaei, H., & Nazeri, M. (2011). Serological evidences of canine brucellosis as a new emerging disease in Iran. Asian *Pacific Journal of Tropical Disease*. 1808(September). https://doi.org/10.1016/S2222-1808(11)60023-6

Alfattli, HHHH (2016). In Iraq, First Documentation of Canine Brucellosis by Application of Three Techniques (Rapid test, Indirect ELISA and 16S rDNA Inter-spacer PCR). مجلة الكوفة للعلوم الطبية البيطرية | *Kufa Journal For Veterinary Medical Sciences*, 7 (2).

Behzadi, M.A., & Mogheiseh, A. (2011). Epidemiological survey of *Brucella canis* infection in different breeds of dogs in Fars Province, Iran. *Pakistan Veterinary Journal*, 32, 234-236.

Bardenstein, S., Waner, T., Etinger, M., Even Tov, B., Blum, S., & Bellaiche, M. (2021). First diagnosis of *Brucella canis* infection in dogs in Israel. *Israel Journal of Veterinary Medicine*, *76*(1), 12–18

Bae, D. H. (2009*)*. Occurrence of canine brucellosis in Korea and polymorphism of isolates by infrequent restriction site-PCR. *Korean Journal of Veterinary Research, 49*, 105–111.

Bastan, I., & Bas, B. (2017). Investigation of the Prevalence of *Brucella canis* in Dogs, Veterinarians and Veterinary Faculty Students in Different Part of Turkey. Turkish Journal of Veterinary Research, *1*(1), 7–11.

Bahaman, A. R. (2006). Investigation of Canine Brucellosis in Klang Valley Malaysia. *Journal of Animal and Veterinary Advances*, *5*(1), 42-44.

Castillo, Y., Tachibana, M., Kimura, Y., Kim, S., Ichikawa, Y., Endo, Y., Watanabe, K., Shimizu, T., & Watarai, M. (2014). Microplate Agglutination Test for Canine Brucellosis Using Recombinant Antigen-Coated Beads. *International Scholarly Research Notices*, *2014*(Ag 4), 1–4. https://doi.org/10.1155/2014/348529

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Ergene, O., Celebi, B., & Kucukaslan, I. (2019). Seroprevalance of canine brucellosis and toxoplasmosis in female and male dogs and relationship to various factors as parity, abortion and pyometra. *Indian Journal of Animal Research*, 53(7), 954-958

Erbas, G., Kirkan, S., & Turkyilmaz, S. (2020). Investigation of The Presence of *Brucella canis* in Dogs in Western Part of Turkey. *Indian Journal of Animal Research*. https://doi.org/10.18805/ijar.B-1228

Hong, J., Park, J., Han, T., & Hwang, H. (2010). Investigation of *Brucella canis* infection in public animal shelters and kennels in Incheon. Korean Journal of Veterinary Research, *33* (1*)*, 23–27

Jamil, T., Melzer, F., Khan, I., Iqbal, M., Saqib, M., Hussain, M. H., Schwarz, S., & Neubauer, H. (2019). Serological and molecular investigation of *Brucella* species in dogs in Pakistan. *Pathogens*, *8*(4). https://doi.org/10.3390/pathogens8040294

Jung, J. Y., Yoon, S. S., Lee, S., Park, J. W., Lee, J. J., Her, M., So, B. J., & Kim, J. H. (2020). Pathological, immunohistochemical, and bacteriological findings in dogs infected with *Brucella canis*. *Korean Journal of Veterinary Research*, *60*(1), 9–14. https://doi.org/10.14405/kjvr.2020.60.1.9

Jung, J. Y., Yoon, S. S., Lee, S. H., Park, J. W., Lee, J. J., Her, M., So, B. J., & Kim, J. H. (2018). Prevalence state of canine brucellosis in South Korea during 2015 and 2016. *Korean Journal of Veterinary Research*, *58*(3). https://doi.org/10.14405/kjvr.2018.58.3.125

Kang, S.I., Lee, S.E., Kim, J.Y., Lee, K., Kim, J.W., Lee, H.K., Sung, S.R., Heo, Y.R., Jung, S.C., Her, M. (2014). A new *Brucella canis* species-specific PCR assay for the diagnosis of canine brucellosis. *Comparative Immunology, Microbiology and Infectious Diseases*. 37(4): 237-241.

Kimura, M., Imaoka, K., Suzuki, M., Kamiyama, T., & Yamada, A. (2008). Evaluation of a Microplate Agglutination Test (MAT) for serological diagnosis of canine brucellosis. *Journal of Veterinary Medical Science*, *70*(7), 707–709. https://doi.org/10.1292/jvms.70.707

Kim, J. W., Lee, Y. J., Han, M. Y., Bae, D. H., Jung, S. C., Oh, J. S., Ha, G. W., & Cho, B. K. (2007). Evaluation of immunochromatographic assay for serodiagnosis of *Brucella canis*. *Journal of Veterinary Medical Science*, *69*(11), 1103–1107. https://doi.org/10.1292/jvms.69.1103

Makloski, C. L. (2011). Canine brucellosis management. Veterinary Clinics: *Small Animal Practice*, 41(6), 1209-1219

Mol, J. P. S., Guedes, A. C. B., Eckstein, C., Quintal, A. P. N., Souza, T. D., Mathias, L. A., Haddad, J. P. A., Paixão, T. A., & Santos, R. L. (2020). Diagnosis of canine brucellosis: comparison of various serologic tests and PCR. *Journal of Veterinary Diagnostic Investigation*, *32*(1), 77–86. https://doi.org/10.1177/1040638719891083

Mosallanejad, B., Ghorbanpoor, N., Avizeh, R., Mohammadian, N., (2009). A serological survey on *Brucella canis* in companion dogs in Ahvaz. *Iranian Journal of Veterinary Research, 10*(4): 383-386

Mosallanejad, B., & Avizeh, R. (2013). *Brucella canis*-induced anterior uveitis in companion dogs in Ahvaz, Iran. *Comparative Clinical Pathology, 23,* 1233-1236. https://doi.org/10.1007/s00580-013-1767-8

Mitra, J., Pal, B., Singha, A., Sarkar, S. N., & Roy, B. B. (2013). Serological diagnosis of Canine Brucelllosis in a kennel of Kolkata, India. *Exploratory Animal and Medical Research*, *3*, 78–80.

Ngamkala, S., Angkawanish, T., Nokkaew, W., & Thongtip, N. (2020). Serological study on brucellosis in captive elephants (*Elephas maximus*) and stray dogs in North Thailand. *Veterinary World*, *13*(9), 1992–1997.

Öncel, T., Akan, M., Sareyyüpoğlu, B., Tel, O. Y., & Çiftci, A. (2005). Seroprevalence of *Brucella canis* infection of dogs in two provinces in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, *29*(3).

Rahman, M. S., Kabir, S. L., & Rahman, M. S. (2015). Seroprevalence of canine brucellosis in Dhaka city corporation area, Bangladesh. *Asian Journal of Medical and Biological Research*, 1(1), 17–21. https://doi.org/10.3329/ajmbr.v1i1.25493

Santos, R. L., Souza, T. D., Mol, J. P. S., Eckstein, C., & Paíxão, T. A. (2021). *Canine Brucellosis : An Update*. *8* (March), 1–17. https://doi.org/10.3389/fvets.2021.594291

SaytekİN, A. M., Karagül, M. S., Eroğlu, B., & Öz, G. Y. (2018). The first-time isolation of *Brucella canis* from two aborted bitches in a kennel in Turkey. *Turkish Journal of Veterinary & Animal Science*, *42*(6), 665–668. https://doi.org/10.3906/vet-1803-54

Sharma, B., Sinha, D. K., & Singh, D. K. (2012). *Seroprevalence of Brucella infection in dogs*. *The Indian Veterinary Journal*, *89*(7), 11-13.

Spickler, A. R., (2018). Brucellosis: Canine Brucellosis. United States Department of Agriculture Animal Plant Health Inspection Service. Retrieved from: http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php.

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Tsogtbaatar, G., Tachibana, M., Watanabe, K., Kim, S., Suzuki, H., & Watarai, M. (2008). Enzyme-linked immunosorbent assay for screening of canine brucellosis using recombinant Cu-Zn superoxide dismutase. *Journal of Veterinary Medical Science*, *70*(12), 1387–1389. https://doi.org/10.1292/jvms.70.1387

Uçan, U.S., Aras, Z., & Zorlutuna, M. (2010). Detection of canine brucellosis by a rapid agglutination test using *Rhizobium tropici* as antigen. *Revue De Medecine Veterinaire*, 161, 51-56.

Ucan, U. S., Aras, Z., & Semacan, A. (2010). Serodiagnosis of *Brucella canis* infection in dogs by a dipstick enzyme immunoassay. *Eurasian Journal of Veterinary Sciences*, 26(2), 109-112.

Wanke, M. M. (2004). Canine brucellosis. *Animal reproduction science*, *82*, 195-207.

Wanke, M. M., Cairó, F., Rossano, M., Laiño, M., Baldi, P. C., Monachesi, N. E., Comercio, E. A., & Vivot, M. M. (2012). Preliminary Study of an Immunochromatography Test for Serological Diagnosis of Canine Brucellosis. *Reproduction in Domestic Animals*, *47*(SUPPL. 6), 370–372. https://doi.org/10.1111/rda.12108

Watarai, M., Kim, S., Yamamoto, J., Miyahara, K., Kazama, M., Matsuoka, S., Chimura, S., & Suzuki, H. (2007). A rapid agglutination assay for canine brucellosis using antigen coated beads. *Journal of Veterinary Medical Science*, *69*(5), 477–480. https://doi.org/10.1292/jvms.69.477

Xiang, F., Xia, Z., Wu, Q., Chen, Y., Yu, J., & Wan, J. (2013). Seroepidemiology of canine brucellosis in Beijing, China. *Turkish Journal of Veterinary and Animal Sciences*, *37*(1). https://doi.org/10.3906/vet-1106-19

Yumusak, N., Polat, P. F., Gurbilek, S. E., Sen, C. C., & Yöntemleri, E. (2017). *A Comparison of the Performance of Exfoliative Cytopathology , Polymerase Chain Reaction (PCR), Culture and ELISA in the Detection of Brucella canis*. *6*(1), 51–5

Zhong, Z., Tu, R., Wang, X., Geng, Y., Xiao, Q., Tian, Y., Wei, B., Dan, J., Wang, Y., & Peng, G. (2019). First Isolation of *Brucella canis* from Pet Dogs in Sichuan Province, China: Molecular Characterization, Pathogenicity and Antigen Location Analysis. *Pakistan Journal of Zoology,* 52(1). https://doi.org/10.17582/journal.pjz/2020.52.1.283.291

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Appendices

Table 4: Number of positive *Brucella canis* cases in dogs in Asia from 2000 to 2021 detected by using different agglutination tests

Country	Year	Total sample	No. of positive cases	References
Central Japan	2007	318	TAT =41/318 (12.89) Latex agglutination test =12/318 (3.77%)	(Watarai et al., 2007)
Japan	2008	485	MAT=12/485(2.5%)	(Kimura et al., 2008)
Japan	2014	743	TAT =9/743 (1.2%) MAT =confirmatory test -SOD = 8/9 (88.9%) -RBP =6/9 (66.7%) -Ag 4 =6/9 (66.7%) -Hsp60 =7/9 (77.8%)	(Castillo et al., 2014)
Korea	2007	463	2ME-RSAT =183 (39.5%)	(Kim et al., 2007)
Korea	2008	224	TAT =30 (13.4%)	(Tsogtbaatar et al., 2008)
Korea	2009	501	2ME-RSAT =53/501 (10.6%)	(Bae, 2009)
India	2012	527	-RBPT =62/527 (11.76%) -2ME-TAT =12/527 (2.27%)	(Sharma et al., 2012)
India	2013	10	2ME-RSAT =5/10 (50%)	(Mitra et al., 2013)
Turkey	2005	362	TAT =46 /362 (12.7%), 2ME-TAT =28/362 (7.73%)	(Öncel et al., 2005)
Konya,Turkey	2010	135	MPAT =34 (25.2%), 2ME-TAT =30 (22.2%) RSAT =33 (24.4%)	(Zeki Aras et al., 2010)
Turkey	2010	135	RAT/RSAT =39/135 (28.9%)	(Zeki Aras, 2010)

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Turkey	2017	1559	MAT =12 (0.8%)	(Bastan & Bas, 2017)
Turkey	2018	2	R-RSAT =2/2 (100%) S-RSAT =0 S-TAT =0 R-2ME-TAT =2/2 (100%)	(SaytekİN et al., 2018)
North Cyprus, Turkey	2019	33	MAT =1/33 (3.03%)	(Ergene et al., 2018)
Turkey	2020	200	2ME-RSAT =12/200 (6%)	(Erbas et al., 2020)
Beijing, China	2013	1200	RBPT =60/1200 (5%) (Confirmed by TAT) =21/1200 (1.75%)	(Xiang et al., 2013)
China	2020	2	RSAT =2/2 (100%) RBPT =2/2 (100%)	(Zhong et al., 2020)
Pakistan	2019	181	MAT/SAT =68/181 (37.6%)	(Jamil et al., 2019)
Bangladesh	2015	50	RBPT =2/50 (4%)	(Rahman et al., 2015)
Malaysia	2006	123	ME-TAT =6/123 (4.8%) RBPT =0	(Khairani-Bejo et al., 2006)
Jordan	2019	169	RSAT =14/169 (8.3%)	(Alshehabat & Obaidat, 2019)
Israel	2021	21	2ME-RSAT =3/21 RBPT =0	(Bardenstein et al., 2021)

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Table 5: Number of positive *Brucella canis* cases in dogs in Asia from 2000 to 2021 detected by using Enzyme-linked immunosorbent assay

Country	Year	Total sample	No. of positive cases	References
Korea	2008	224 (30)	i-ELISA =30/30 (100%) Western blotting in ELISA =26/30 (86.7%)	(Tsogtbaatar et al., 2008)
India	2012	527	Dot-ELISA =16/527 (3.03%)	(Sharma et al., 2012)
Turkey	2005	362	i-ELISA =27 (7.45%)	(Öncel et al., 2005)
Konya, Turkey	2010	135	I-ELISA =29 (21.5 %)	(Öncel et al., 2005)
Turkey	2010	135	i-ELISA =33/135 (24.4%) Dipstick EIA =32/135 (23.7%)	(Zeki Aras, 2010)
Turkey	2017	147	i-ELISA (serum) =13/147 (11.5%)	(Yumusak et al., 2017)
Thailand	2020	16	Dot-ELISA =2/16 (12.5%)	(Ngamkala et al., 2020)
Bangladesh	2015	50	i-ELISA =2/50 (4%)	(Rahman et al., 2015)
Iraq	2016	243	I-ELISA =31 (12.76%)	(Hams et al., 2016)
Israel	2021	21	Dot-ELISA =3/21 (14.29%)	(Bardenstein et al., 2021)

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Table 6: Number of positive *Brucella canis* cases in dogs in Asia from 2000 to 2021 detected by immunochromatography assay

Country	Year	Total sample	No. of positive cases	References
Korea	2007	463	ICA =181 (39.1%)	(Kim et al., 2007)
Korea	2008	402	ICA =10/402 (2.5%)	(Hong et al., 2010)
Korea	2009	501	ICA =25/501 (5.0%)	(Bae, 2009)
Korea	2018	2394	ICA =30 (1.3%)	(Jung et al., 2018)
India	2013	10	ICA =5/10 (50%)	(Mitra et al., 2013)
Iran	2013	243	ICA =16/243 (6.58%)	(Mosallanejad et al., 2013)
Fars province,Iran	2011	113	ICA =12 (10.61%)	(Behzadi & Mogheiseh, 2011)
Iraq	2016	243	ICA =14 (5.76%)	(Hams et al., 2016)

Table 7: Number of positive *Brucella canis* cases in dogs in Asia from 2000 to 2021 detected by using Polymerase Chain Reaction

Country(Asia)	Year	Total sample	Samples	No. of positive cases	References
Central Japan	2007	318	Blood	PCR in TAT =5/12 (47.1%)	(Watarai et al., 2007)
Konya, Turkey	2010	48	Lymph node	PCR =4/48 (8.3%)	(Aras & Uçan, 2010)
Turkey	2017	110	Vaginal and preputial swabs	PCR =2/110 (1.8%)	(Yumusak et al., 2017)
Turkey	2018	2	Vaginal and blood sample	-Bcsp31 PCR (3 strain) =2/2 -Multiplex PCR =2/2	(Saytekİn et al., 2018)

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Turkey	2020	200	Blood sample	BcSS PCR =10/200(5%)	(Erbas et al., 2020)
China	2020	2	Blood samples	BcSS-PCR =2/2 BCSP31 PCR =2/2	(Zhong et al., 2020)
Iraq	2016	243	Blood samples	PCR =5/31 (16.13%)	(Hams et al., 2016)
Israel	2021	5	Blood samples	PCR =5/5 (100%)	(Bardenstein et al., 2021)

Table 8: Number of positive *Brucella canis* cases in dogs in Asia from 2000 to 2021 detected by bacterial culture and isolation

Country	Year	Total sample	Sample	No. of positive cases	References
Korea	2007	463	Whole Blood	Hemoculture =115(24.8%)	(Kim et al., 2007)
Korea	2008	402	Whole blood	Bacterial culture =5/10 (50%)	(Hong et al., 2010)
Korea	2009	501	Whole blood	Bacterial culture =4/501 (0.8%)	(Bae, 2009)
Korea	2018	2394	Whole blood	Bacterial culture =2/196 (1%)	(Jung et al., 2018)
Korea	2020	40	Whole blood Tissue	Bacterial culture =33 (82.5%)(died) Bacterial culture = a variety	(Jung et al., 2020)
Turkey	2010	48	Tissue Lymph node	Bacterial culture =4/48 (died)	(Aras & Uçan, 2010)
Turkey	2018	2	Vaginal swabs and blood samples	Bacterial isolation =2/2 Bacterial isolation =2/2	(SaytekİN et al., 2018)

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China	2020	2	Blood samples	Bacterial isolation =2/2 (W5 and Y4)	(Zhong et al., 2020)
Israel	2021	1 7	Blood samples Vaginal swabs	Bacterial isolation =1 Bacterial isolation =1/7	(Bardenstein et al., 2021)
Malaysia	2006	123	Blood sample	0 isolation	(Khairani-Bejo et al., 2006)
Turkey	2017	147	Vaginal and preputial swab	0 isolation Were not isolated	(Yumusak et al., 2017)

Table 9 : Number of *positive Brucella canis* cases in dogs in Asia from 2000 to 2021 detected by agar gel immunodiffusion assay and immunofluorescence assay

Country	Year	Total sample	No. of positive cases	References
India	2012	527	AGID =8/527 (1.5%)	(Sharma et al., 2012)
Israel	2021	21	AGID =3/21 (14.2%)	(Bardenstein et al., 2021)
Kerman, Iran	2011	95	IFA =15 (15.8%)	(Akhtardanesh et al., 2011)

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Appendix 1:

Calculation for seropositive of the *Brucella canis* infection in dogs in Asia by using agglutination test:

$$= \text{TAT} + \text{2ME-TAT} + \text{MAT} + \text{RSAT} + \text{2ME-RSAT} + \text{RBPT} + \text{LAT}$$

$$= \frac{147 + 78 + 135 + 76 + 270 + 126 + 12}{2849 + 1149 + 2402 + 464 + 1364 + 1923 + 318}$$

$$= \frac{844}{10469} \times 100$$

$$= 8.1\%$$

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