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Review Article

Presence of *Brucella* spp. in Milk and Dairy Products: A Comprehensive Review and Its Perspectives

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Consuming raw milk and milk-based products that have not been produced under strict control conditions can cause brucellosis, a highly contagious zoonotic disease. It is a significant global public health concern, particularly in regions with poor management and limited resources, such as Latin America, North and East Africa, the Middle East, and South and Central Asia. The study aims to summarize the occurrence of human brucellosis linked to milk and milk products and the presence of *Brucella* species in dairy foods. To achieve this goal, a meta-analysis was conducted on 69 studies ranging from 2001 to 2022, which were categorized into two groups: the incidence of *Brucella* species in milk and milk products and the prevalence of human brucellosis resulting from the consumption of contaminated milk. The following milk and milk products showed the highest incidence of *Brucella* species: cow milk (1.86%–81.7%), buffalo milk (10.4%–61.67%), camel milk (0%–24%), goat milk (0%–88.8%), and cheese (0%–39.1%). Consuming unpasteurized milk and milk products has been identified as the leading cause of human brucellosis, with incidence rates varying from 33.9% to 100%. Several human brucellosis cases have been linked to consuming raw milk and cheese in Spain, Israel, and other countries. Various serological techniques are employed to detect *Brucella*-specific antibodies in milk. The milk ring test (MRT) and enzyme-linked immunoassay (ELISA) are the two most widely utilized methods for detecting these antibodies in milk. Recently developed dual biosensors are a powerful approach for early diagnosis of *Brucella* from milk. Real-time PCR can rapidly detect organisms, reducing the risk of lab contamination and false positive results. To prevent and control brucellosis, essential steps include proper pasteurization of milk and dairy products, using the milk ring test (MRT) to detect *Brucella* in individual and bulk milk, immunization, education, and increasing public awareness of the disease. The consumption of raw milk and milk-made products that are not produced under strictly controlled conditions poses a significant risk to human health, mainly due to the high incidence of *Brucella* contamination. Therefore, ensuring strict control measures in producing milk and milk-made products is crucial to preventing the spread of this disease and safeguarding human health.

1. Introduction

Brucellosis is an often overlooked zoonotic disease, primarily due to its nonspecific symptoms and lack of awareness among healthcare professionals [1]. The most frequently occurring species responsible for human infection include *B. abortus, B. melitensis*, and *B. suis* [2], while *B. canis* also poses a risk of brucellosis in humans [3]. The illness, which is often referred to as Mediterranean fever or Malta fever, can present with symptoms such as undulant fever, weight loss, night sweats, and increasing weakness and may also cause abortion in infected women [4]. Those most at risk of *Brucella* infection include livestock owners, caterers, artificial inseminators, milkers, vets, and laboratory employees [5, 6]. The dairy sector experiences significant economic losses due to brucellosis, as the disease can lead to miscarriage, infertility, stillbirths, and decreased milk production.

Brucella can be transmitted from animals to humans through various routes. First, it can be present in unpasteurized milk, dairy products, and undercooked meat from infected animals. Second, direct exposure to diseased animals can also result in transmission. Third, *Brucella* can be present in dust and other airborne particles where infected animals have been kept or slaughtered. People who inhale these particles can become infected with the bacteria. Fourth, researchers who work with *Brucella* in laboratories may accidentally expose themselves to the bacteria through cuts or other skin wounds. *Brucella* can also be transmitted through direct contact with aborted materials, venereal contact (though rare), blood transfusions, and tissue transplantation. Finally, in areas where *Brucella* is common, individuals may be exposed to the bacteria through direct or indirect contact with their mouth, eyes, or respiratory system [7–11].

Brucella spp. predominantly infects the reproductive system of animals as this is where erythritol, a sugar alcohol, is abundantly present. Erythritol serves as a critical nutrient for *Brucella* spp., allowing them to replicate and establish an infection in the reproductive organs of animals [12]. The destruction of maternal placental cells and the accumulation of exudates between the maternal and fetal parts of the placenta caused by *Brucella* species during the third trimester of pregnancy can result in fetal death and abortion [13]. The third trimester of gestation is typically the time when female animals generally abort but further uterine invasion starts with the next pregnancy, including the shedding of embryonic fluid and fetal membranes. In the later stage of infection, the disease may be characterized by the organism released through the mammary gland and vaginal fluids [14].

According to a study by Dadar et al. [15], countries with lower gross domestic product (GDP) exhibited a higher prevalence of *Brucella* species in milk and dairy products compared to other countries, while the Western Pacific region had a lower incidence (15.32%) and Southeast Asia had a higher incidence (25.55%) of *Brucella* spp. Contamination of raw milk and milk products is a significant concern for public health in developing nations. In contrast, developed countries have implemented strict regulations and control measures, such as mandatory testing and vaccination programs, to keep their animals free from brucellosis [16]. Raw milk and milk products from various animals (cows, goats, sheep, donkeys, buffaloes, yaks, and camels) are increasingly popular in developing countries due to their affordability, availability, cultural, and traditional preferences. However, this trend raises concerns about potential foodborne illnesses linked to unpasteurized dairy products [17].

According to several studies, goat milk had the highest prevalence of *Brucella* species, whereas camel milk and traditional cheese made from cow and goat exhibited the lowest prevalence rates [18, 19]. The rates of human brucellosis were higher among Iranian and Israeli consumers who consumed unpasteurized dairy products, including raw milk [20–22].

Accurate diagnosis of brucellosis in farm animals remains one of the biggest obstacles to completely eradicating the disease [23]. Serological methods, which detect *Brucella* antibodies, can be used for brucellosis detection [24]. Two methods, the milk ring test (MRT) and indirect ELISA (iELISA), are suitable for detecting *Brucella*-specific antibodies in milk. However, the gold standard technique for diagnosing *Brucella* spp. is the isolation of *Brucella* from clinical samples taken from an affected animal [25]. The standard methods for isolating *Brucella* from clinical and nonclinical specimens involve using enriched media such as Farrell's, Castaneda's, and modified Thayer–Martin media. These media are supplemented with selective agents such as antibiotics and dyes to inhibit other microorganism's growth and enhance *Brucella* development. The inoculated media are then incubated at 37°C with 5–10% CO_2 for 7–21 days, and colonies are identified based on their characteristic morphology, growth rate, and agglutination properties [1]. The species of *Brucella* can be identified at the genus, species, and biovar levels by various bacteriological methods and traditional biotyping systems [26, 27]. The traditional biotyping system of *Brucella* categorizes the bacteria based on their ability to grow in specific culture media containing various carbon sources and dyes as well as their sensitivity to dyes and antibiotics [28]. A recent study reported that *Brucella* species could be identified at the genus, species, and biovar levels by the PCR test [28]. PCR is a sensitive and specific method that can identify *Brucella* spp. The genomic regions targeted in PCR tests for *Brucella* detection may vary depending on the specific assay used. However, several studies have reported using different targets, such as the *Brucella*-specific insertion sequence *IS711*, the *bcsp31* gene, and the *omp2a* and *omp2b* genes. The *IS711* insertion sequence is the most commonly used target in PCR assays for *Brucella* detection. There are many copies of this sequence present in the Brucella genome and absent in other bacteria, making it a specific and sensitive target. The primers used to amplify *IS711* can be designed to target specific *Brucella* species, allowing for differentiation between them.

Brucella can infect and replicate within several different types of host cells, including macrophages, dendritic cells, epithelial cells, and trophoblasts. However, the primary target cells for *Brucella* infection are the macrophages, which are the cells of the immune system that engulf and destroy invading pathogens. The pathogenesis of *Brucella* within

host cells involves a series of steps. First, the bacteria adhere to and are internalized by host cells through phagocytosis. Once inside the host cell, *Brucella* uses various mechanisms to prevent being destroyed by host defenses, such as avoiding fusion with lysosomes and altering the trafficking of host cell membranes. *Brucella* then begins to replicate within the host cell, using nutrients and resources from the host to support its growth. As the bacteria multiply, they can cause damage to the host cell, leading to cell death and the release of bacteria into the surrounding tissue. *Brucella* can also modulate host immune responses, which can contribute to its ability to establish a persistent infection. By dampening host immune responses, *Brucella* can avoid detection and clearance by the host's immune system, allowing it to survive within the host for prolonged periods. Due to their intracellular nature and tendency to cause late infection, the antibody titers against *Brucella* species may fall below the diagnostic threshold. As a result, animals with an undetected *Brucella* infection can potentially release *Brucella* organisms into their milk, which can threaten human health [29–31].

Detecting *Brucella* can be a complex process due to various factors. Identifying *Brucella* species in the body often requires multiple serological tests [1]. Accurate diagnosis and identification of *Brucella* species biovars are crucial for effectively preventing and controlling measures. These measures involve timely treatment with antibiotics, vaccination of livestock, strict hygiene during animal product processing, and public health education to reduce transmission. Prioritizing these measures is necessary to mitigate the impact of brucellosis on human and animal health [32–34]. Due to the absence of a dependable diagnostic test and the limited availability of targeted therapies for severe human brucellosis, it is crucial to prioritize general care and manage endemic areas to prevent brucellosis [16]. The milk ring test (MRT) is a commonly used diagnostic method for detecting the presence of *Brucella* species in milk samples. It is a rapid, simple, and inexpensive test that can be performed onsite, making it ideal for use in field settings or areas with limited resources. The MRT works by detecting antibodies produced by the animal's immune system in response to *Brucella* infection, which is present in the milk. By identifying the infected animals early, farmers can prevent the spread of brucellosis within their herds and reduce the risk of transmission to humans. Moreover, some countries have implemented immunization programs for domestic animals against brucellosis, which could be a critical control measure for improving both animal and human health. The immunization programs include vaccinating susceptible livestock populations. The most commonly used brucellosis vaccines include live-attenuated and killed vaccines. Live-attenuated vaccines like RB51 provide high protection and reduce brucellosis incidence but may cause adverse reactions and affect diagnostic testing. In contrast, killed vaccines like S19 are safer and do not interfere with diagnostic testing but may provide lower protection [35].

The main focus of this review is to analyze the current situation regarding the occurrence of brucellosis and investigate approaches that can be employed to reduce the incidence of human brucellosis resulting from milk and milk products. Moreover, the review explores diverse techniques that can precisely diagnose *Brucella* species from milk samples. The study highlights the importance of identifying and executing effective measures for preventing and controlling brucellosis.

2. Methodology

A comprehensive literature search was conducted to perform a review. The search encompassed various databases, including PubMed, Google Scholar, AGORA, and HINARI, and employed medical subject headings (MeSHs) terms, such as brucellosis, milk, milk products, diagnosis, and prevention. The review focused solely on English-language articles related to brucellosis and milk published between 2001 and 2022, using the following criteria to determine which studies were included: (1) A complete English text; (2) the presence of *Brucella* contamination in milk and dairy products; (3) original studies on the incidence of human brucellosis following the consumption of raw milk and other milk-made products; and (4) studies describing the presence of *Brucella* species in milk and other dairy products and the detection techniques used. Articles that did not meet any of these criteria were excluded. The information gathered from the publications included the incidence of brucellosis resulting from consuming contaminated dairy products, different diagnostic techniques for detecting *Brucella* in milk and other milk products, and effective preventive measures. A meta-analysis was conducted on the data from 69 trials evaluated in two categories, using MedCalc® statistical software version 20.106 to ensure the accuracy of the presented information. MedCalc® offers several options for managing data for meta-analysis, including importing data from various sources, cleaning data by removing duplicates, identifying outliers and imputing missing values, and calculating different effect sizes (SMD, RR, and OR) and statistical methods (fixed-effect models and random-effects models) for meta-analyses. It also has tools for assessing publication bias, such as funnel plots and Egger's regression test. The meta-analysis output included a forest plot that graphically represented the findings, displaying each study's effect sizes and confidence intervals, along with the overall effect size and corresponding confidence interval. MedCalc® also provided statistics for assessing heterogeneity, such as the Q-statistic and I-squared statistics. Using random-effects meta-analyses, we calculated the prevalence of *Brucella* species in milk and other dairy products and human brucellosis caused by drinking milk and dairy products. We used *I*-squared to evaluate statistical heterogeneity across trials and Egger's test to identify publication bias [36].

3. Key Findings

3.1. Brucella Prevalence in Milk and Various Dairy Products. Human brucellosis is mainly caused by consuming raw or insufficiently boiled cow milk, particularly in rural areas [37]. The prevalence of *Brucella* species in cow milk varies across countries, with Turkey recording the highest prevalence rate at 81.7%, while the lowest rate was 1.86%. According to Tables 1 and 2, the average prevalence rate of Turkey between 2013 and 2018 was 13.24%.

Table 1: *Brucella* prevalence in milk and dairy products.

Geographical areas (period)	Types of sample (*n*)	Number of positive samples	Prevalence (%)	Average prevalence (%)	*Brucella* spp.	Methods of detection	References
Azerbaijan (2022)	Cow milk (57)	30	52.63	52.63	*B. abortus*, *B. melitensis*	Culture	[38]
Bangladesh (2018-2019)	Cow milk (360)	24	6.6	4.17	Not determined	Culture, PCR	[1]
	Cow milk (115)	2	1.73		*B. abortus* biovar 3	Culture, PCR	[28]
Brazil (2017)	Cow raw milk (15)	0	0	6.6	Not determined	PCR	[39]
	Fresh cheese (38)	5	13.2		Not determined		
China (2012-2013)	Sheep raw milk (110)	7	6.4	3.75	Not determined	LAMP	[40]
	Cow milk (5211)	57	1.1		Not determined	PCR	[41]
Egypt (2008–2014)	Cow raw milk (32)	6	18	17.28	*B. melitensis*	PCR followed by culture	[42]
	Buffalo raw milk (30)	4	13				
	Sheep raw milk (30)	6	20				
	Cow milk (72)	18	25		*Brucella* spp.	iELISART-PCR	[43]
	Buffalo raw milk (125)	13	10.4				
India (2006–2021)	Goat milk (54)	48	88.8	25.76	*B. abortus*	PCRMRT	[18]
	Cow milk (483)	21	4.4		Not determined	MRT	[44]
		28	5.8			ELISA	
	Cow milk (174)	31	17.9		*Brucella* spp.	ELISA	[45]
	Buffalo milk (96)	11	11.9		*Brucella* spp.	ELISA	
Iran (2010–2018)	Raw milk of cow (120)	73	60.8	21.58	*B. abortus*	PCR followed by culture	[46]
	Traditional cheese (1000)	22	2.2		*B. melitensis*	MRT and ELISA	[47]
	Raw cow milk (60)	33	53.3		*Brucella* spp.	PCR followed by culture	[48]
	Cow milk (132)	4	3		*B. melitensis*	PCR followed by culture	[49]
	Cow raw milk (139)	4	2.8		*B. abortus*	Biochemical and serological screening test	[50]
	Sheep raw milk (50)	1	2				[50]
	Sheep raw milk (125)	12	9.6		*B. melitensis*		[51]
	Goat raw milk (100)	18	18		Not determined		
	Sheep raw milk (330)	18	5.5		Not determined	PCR	[52]
	Goat raw milk (470)	51	10.9				
	Sheep raw milk (300)	3	1		*B. ovis*	PCR followed by culture	[53]
	Goat raw milk (400)	6	1.5		*B. melitensis*biovar 1		
	Cow raw milk (120)	12	10		*B. abortus*	PCR followed by culture	[54]
Iran (2010–2018)	Sheep raw milk (33)	9	27.3		Not determined	RT-PCR	[55]
	Cow raw milk (57)	15	26.3				
	Goat milk (33)	15	45.5				
	Pasteurized or treated milk (34)	5	14.7				
	Pasteurized or treated cheese (28)	7	25				
	Unpasteurized cheese (23)	9	39.1				

Table 1: Continued.

Iraq (2009–2016)	Cow milk (120)	29	24.2	7.51	*B. abortus*	MRT	[56]
	Ice cream traditional (100)	0	0				[57]
	Raw sheep milk (50)	34	68		*B. melitensis*	PCR followed by culture	[58]
	Sheep raw milk (60)	30	50		Not determined	iELISART-PCR	[59]
Israel (2021)	Camel milk (34)	4	11.76	11.76	*B. melitensis*	CulturePCR	[60]
Italy (2008)	Buffalo raw milk (60)	37	61.67	61.67	*Brucella* spp.	ELISAPCR	[61]
Kuwait (2016)	Cow raw milk (60)	37	61.7	61.7	*B. melitensis* biovar 2	MRT	[62]
Nigeria (2017)	Cow raw milk (57)	19	33.3	13.4	Not determined	MRT	[63]
		2	3.5			iELISA	
	Cow raw milk (174)	6	3.4		*B. abortus*	MRT and ELISA	[64]
Pakistan (2011)	Raw cow milk (86)	4	4.6	5.45	Not determined	MRT	[65]
	Raw buffalo milk (114)	2	1.7				
Qatar (2002)	Cow milk (52)	29	55.7	43.9	*B. melitensis*	Culture,PCR	[66]
	Sheep milk (21)	10	47.6				
	Goat milk (18)	13	72.3				
	Camel milk (12)	0	0				
Saudi Arabia (2017)	Camel raw milk (80)	19	24	24	*B. melitensis*	Serological (MRT, ELISA) and PCR	[67]
Syria (2015)	Cow milk (2372)	596	25	25	*B. melitensis*	PCR followed by culture	[68]
Tajikistan (2017)	Cow milk (564)	58	10.3	10.3	*B. melitensisB. abortus*	RT-PCR	[69]
Tanzania (2015)	Cow raw milk (63)	4	6.3	6.3	*B. abortus*biovar 3	ELISA	[6]
Turkey (2013–2018)	Cow raw milk (202)	35	17.3	13.24	*B. abortus*	Biochemical and serological test	[70]
	Cow raw milk (215)	4	1.86		*B. abortus*	PCR	
	Traditional cheese (50)	0	0				[19]
	Butter (50)	0	0			PCR	
	Cow milk (48)	9	18.75		*B. abortus*	iELISART-PCR	[71]
	Sheep milk (65)	4	6.1		*B. melitensis*		
	Goat milk (65)	5	7.6				
	Traditional unpasteurized cheese (80)	18	22.5				[71]
	Traditional cheese (60)	0	0		—	MRT and ELISA	[72]
Turkey	Cow raw milk (334)	273	81.7		*B. abortus*	PCR followed by culture	[73]
	Traditional unpasteurized cheese (200)	12	6		*B. melitensisB. abortus*	MRT and ELISA	[74]
	Traditional cheese (200)	12	6		*B. melitensisB. abortus*	MRT and ELISA	[75]
Uganda (2016-2017)	Cow milk (324)	21	6.5	29.83	*B. abortus*	Real-time PCR	[76]
	Cow milk (185)	62	33.5		Not determined	MRT	[77]
		92	49.5		Not determined	iELISA	
Western Europe (2019)	Cheese (200)	20	10	10	*Brucella* spp.	Real-time PCR	[78]

Table 2: Meta-analysis proportion for the prevalence of brucellosis in milk and dairy products.

Studies/References	Geographical areas	Sample sizes	Proportion (%)	95% CI	Weight (%)	
					Fixed	Random
[38]	Azerbaijan (2022)	57	52.632	38.965–66.015	0.33	1.43
[28]	Bangladesh (2018-2019)	115	1.739	0.211–6.141	0.66	1.48
[1]		360	6.667	4.318– 9.757	2.04	1.50
[39]	Brazil (2017)	38	13.158	4.414– 28.086	0.22	1.40
[40]	China (2012-2013)	110	6.364	2.597–12.673	0.63	1.47
[41]		5211	1.094	0.829–1.415	29.49	1.52
[42]	Egypt (2008–2014)	32	18.750	7.208–36.439	0.19	1.38
		30	13.333	3.755–30.722	0.18	1.37
		30	20.000	7.714–38.567	0.18	1.37
[43]		72	25.000	15.539–36.603	0.41	1.45
		125	10.400	5.655–17.128	0.71	1.48
[44]	India (2006–2021)	483	4.348	2.711–6.570	2.74	1.51
		483	5.797	3.886–8.270	2.74	1.51
[45]		174	17.816	12.438–24.325	0.99	1.49
		96	11.458	5.861–19.578	0.55	1.47
[18]		54	88.889	77.369–95.812	0.31	1.43
[46]	Iran (2010–2018)	1000	2.200	1.384–3.312	5.66	1.51
		120	60.833	51.504–69.614	0.68	1.48
[48]		60	55.000	41.612–67.878	0.35	[64]
[49]		132	3.030	0.832–7.577	0.75	1.48
[50]		139	2.878	0.790–7.204	0.79	1.48
		50	2.000	0.0506–10.647	0.29	1.42
[51]		125	9.600	5.059–16.169	0.71	1.48
		100	18.000	11.031–26.948	0.57	1.47
[63]		330	5.455	3.264–8.484	1.87	1.50
		470	10.851	8.187–14.020	2.67	1.51
[53]		300	1.000	0.207–2.894	1.70	1.50
		400	1.500	0.552–3.236	2.27	1.51
[54]		120	10.000	5.275–16.817	0.68	1.48
[55]		33	27.273	13.300–45.524	0.19	1.38
		57	26.316	15.538–39.663	0.33	1.43
		33	45.455	28.107–63.649	0.19	1.38
		34	14.706	4.953–31.057	0.20	1.38
		28	25.000	10.691–44.872	0.16	1.36
		23	39.130	19.708–61.458	0.14	1.33
[56]	Iraq (2009–2016)	120	24.167	16.821–32.829	0.68	1.48
[58]		50	68.000	53.301–80.480	0.29	1.42
[59]		60	50.000	36.806–63.194	0.35	1.44
[60]	Israel (2021)	34	11.765	3.300–27.450	0.20	1.38
[61]	Italy (2008)	60	61.667	48.211–73.929	0.35	1.44
[62]	Kuwait (2016)	60	61.667	48.211–73.929	0.35	1.44
[63]	Nigeria (2017)	57	33.333	21.401–47.065	0.33	1.43
		57	3.509	0.428–12.107	0.33	1.43
[64]		174	3.448	1.276–7.354	0.99	1.49
[65]	Pakistan (2011)	86	4.651	1.282–11.483	0.49	1.46
		114	1.754	0.213–6.194	0.65	1.47
[66]	Qatar (2002)	52	55.769	41.328–69.530	0.30	1.43
		21	47.619	25.713–70.219	0.12	1.32
		18	72.222	46.520–90.305	0.11	1.29
[67]	Saudi Arabia (2017)	80	23.750	14.945–34.578	0.46	1.46
[68]	Syria (2015)	2372	25.126	23.391–26.923	13.43	1.52
[69]	Tajikistan (2017)	564	10.284	7.902–13.091	3.20	1.51
[6]	Tanzania (2015)	63	6.349	1.757–15.466	0.36	1.44

Table 2: Continued.

Studies/References	Geographical areas	Sample sizes	Proportion (%)	95% CI	Weight (%)	
					Fixed	Random
[70]	Turkey (2013–2018)	334	81.737	77.167–85.732	1.90	1.50
[74]		200	6.000	3.138–10.246	1.14	1.49
[75]		200	6.000	3.138–10.246	1.14	1.49
[71]		48	18.750	8.950–32.629	0.28	1.42
		65	6.154	1.702–15.013	0.37	1.44
		65	7.692	2.545–17.046	0.37	1.44
		80	22.500	13.914–33.209	0.46	1.46
[70]		202	17.327	12.375–23.264	1.15	1.49
[19]		215	1.860	0.509–4.695	1.22	1.49
[76]	Uganda (2016-2017)	324	6.481	4.057–9.737	1.84	1.50
[77]		185	33.514	26.756–40.809	1.05	1.49
		185	49.730	42.310–57.158	1.05	1.49
[78]	Western Europe (2019)	200	10.000	6.216–15.021	1.14	1.49
Total (fixed effects)	—	17603	9.459	9.032–9.900	100.00	100.00
Total (random-effects)	—	17603	21.210	16.115–26.799	100.00	100.00

Buffalo brucellosis poses a substantial challenge for buffalo herds globally. Data from Tables 1 and 2 reveal that Italy has the highest occurrence of *Brucella* in buffalo milk at a staggering 61.67%, while Egypt experiences the lowest prevalence [79]. From 2008 to 2014, the average prevalence of brucellosis in Egypt was 17.28%.

It has been reported that the consumption of camel milk is a significant factor in human brucellosis in the Middle East [80]. According to species, Tables 1 and 2, Saudi Arabia has the highest prevalence of *Brucella* species in uncooked camel milk at 24%, while Qatar has the lowest at 0%.

Brucella species infection continues to be a major concern in several countries, including Saudi Arabia, Iran, Kuwait, India, and several South European countries, with sheep and goat milk being the primary sources of infection [16, 81]. According to a recent study, goat milk consumers are at a higher risk of contracting brucellosis [82]. The incidence of *Brucella* species in goat milk was found to be the highest in India (88.8%), while South Africa had the lowest occurrence (0%). In India, the prevalence percentage of *Brucella* species in goat milk from 2006 to 2021 was 25.76% (Tables 1 and 2). Similarly, the prevalence of *Brucella* species in sheep milk was highest in Iraq (50%) and lowest in Iran (1%). The average prevalence in Iran from 2010 to 2018 was 21.58% (Tables 1 and 2).

Jansen et al. [78] conducted a study that examined 200 cheese samples collected from 13 countries, including Bulgaria, Belgium, Cyprus, Germany, Croatia, Greece, Italy, the Czech Republic, France, Lebanon, the Netherlands, Spain, and Turkey. The results indicated that the prevalence of *Brucella* in the samples was 20.5%. In contrast, the prevalence rate of *Brucella* species in cheese was 39.1% in Iran, while Brazil and Turkey had a prevalence rate of 0%, as shown in Tables 1 and 2.

It is important to note that *Brucella* species can contaminate cheese during production, handling, and storage, particularly if the milk used is contaminated or the cheese is not handled aseptically [17, 43, 83–86]. Furthermore, certain types of stored-ripened cheese are linked to *Brucella* species infections. People can contract *Brucella* species infections by consuming undercooked dairy products such as milk, butter, ice cream, whey, and yogurt [87]. It is worth noting that ice cream made from raw milk is a significant cause of *Brucella* infections [88].

The survival of *Brucella* species in milk depends on various factors such as temperature, pH, and the presence of disinfectants or antimicrobials. When refrigerated at 39°F, raw or unpasteurized milk can harbor the bacteria for several days to weeks [1]. In a study by Zúñiga Estrada et al. [89], *B. abortus* was found to survive in milk for up to 22 days when refrigerated at 39°F and fermented with a yogurt starter culture. The butter kept at 46.5°F can host *Brucella* microorganisms for 142 days. Similarly, the bacteria can persist in ice cream stored at 32°F for up to 30 days and in cheese at room temperature for up to 18 days [1, 90]. Additionally, Kaden et al. [91] reported that *Brucella* might survive in milk for 132 days under various storage conditions.

Brucellosis in domestic animals has been successfully eliminated in most industrialized areas of Northern Europe, New Zealand, Canada, and Australia. However, *Brucella* reservoirs still exist in the wild [16, 92]. The recent studies conducted in India, Iran, and Qatar using PCR techniques have confirmed the presence of *Brucella* species in milk and other dairy products [18, 55, 66]. This is a significant concern as humans can contract *Brucella* infection by consuming contaminated milk (Table 1). Many countries, including India, Iran, Iraq, Kuwait, Qatar, Turkey, and Uganda, have reported a high prevalence of *Brucella* species in dairy products. In 2022, the reported prevalence rate of *Brucella* species in Azerbaijan was 52.63%. The average prevalence of *B. abortus* in Bangladesh from 2018 to 2019 was 4.17%. Iraq had an average prevalence rate of 7.51% from 2009 to 2016, while China had a prevalence rate of 3.75% from 2012 to 2013. In 2017, the reported prevalence rate was 6.6% (Table 1). The prevalence of *Brucella* species was found to be highest in certain milk and milk products. Cow milk showed a range of 1.86%–81.7% incidence, while buffalo milk ranged from 10.4% to 61.67%. Camel milk had a lower range of 0%–

24%, and goat milk showed a 0%–88.8% range. Cheese also showed the prevalence, ranging from 0% to 39.1%. The detection rate of brucellosis in milk and milk products can vary significantly depending on the geographic location, which may be attributed to differences in testing methods. Several factors may impact the detection rate, including the study area, sample size, age, breed, herd size, management practices, sanitary conditions, breeding practices, reproductive illnesses, the animal's pregnancy status, and diagnostic procedures [1, 16]. Therefore, it is essential to consider these factors when interpreting brucellosis detection rates to ensure accurate and reliable results. The heterogeneity in the prevalence of *Brucella* spp. in various milk and dairy products (Table 3) is high at 98.54%. The presence of significant publications was shown by Egger's test (Table 4) with a p value of less than 0.01.

Table 3: Test for heterogeneity for the prevalence of *Brucella* spp.

Q	4669.6156
DF	68
Significance level	$P < 0.0001$
I^2 (inconsistency)	98.54%
95% CI for I^2	98.39–98.68

Table 4: Publication bias test for the study related to the prevalence of *Brucella* spp.

Egger's test	
Intercept	5.8220
95% CI	2.9724 to 8.6717
Significance level	$P = 0.0001$
Begg's test	
Kendall's Tau	0.3102
Significance level	$P = 0.0002$

3.2. Prevalence of Human Brucellosis due to the Intake of Milk and Other Dairy Products. Brucellosis is a severe illness acquired by humans through consuming contaminated milk and dairy products [93]. Numerous studies have been conducted to determine the primary risk factors associated with human brucellosis. It has been consistently found that consuming raw, unpasteurized milk and fresh cheese is a major contributor to the illness [94–97]. Previous research has also confirmed that raw or undercooked milk is the leading cause of human brucellosis. For instance, a study conducted in Malaysia identified 79 cases of human brucellosis. All affected individuals had a history of consuming unpasteurized milk, resulting in an average incidence rate of 87.3% [98]. Similarly, a study in Turkey revealed that 63.6% of the 1028 patients with brucellosis had consumed unpasteurized milk or milk products [99]. For seven years (2003–2010), Turkey saw an average prevalence rate of 63.6% for human brucellosis (Tables 5 and 6). Similarly, Spain saw an average prevalence rate of 100% for human brucellosis from 2003 to 2011 [108]. An observation by Mendez Martinez et al. [108] identified that eleven cases of human brucellosis were caused by consuming cheese made from raw goat milk. Moreover, several other studies have emphasized that drinking raw milk is the leading cause of human brucellosis [4, 20, 101–107]. A 2021 study in Ethiopia indicated that the prevalence of human brucellosis resulting from unpasteurized milk was 33.9%, whereas, in West Palestine, the prevalence was 59.05%. Similarly, in Oman in 2001, the prevalence percentage was 63% among the 375 individuals who participated in the study. Tables 5 and 6 show that the average prevalence rate of human brucellosis in Iran from 2004 to 2016 was 69.55%.

Consuming contaminated milk, cheese, and butter has been found to spread brucellosis to individuals [113, 114]. In Spain, Ramos et al. [109] reported three cases of human brucellosis infections linked to consuming raw milk and unpasteurized fresh cheese. Colmenero et al. [110] also identified cases of human brucellosis connected to unpasteurized cheese consumption. Two extensive investigations [21, 22] in Israel have revealed that the individuals diagnosed with human brucellosis had consumed unpasteurized milk and cheese products. In Israel, the highest incidence of brucellosis in humans from 2012 to 2018 was caused by ingesting unpasteurized cheese (100%) and untreated milk (100%). Meanwhile, in 2017, the prevalence of human brucellosis in Rwanda was 6.1%. The prevalence of human brucellosis exhibited a high degree of heterogeneity, with a score of 99.21% (Table 7). After carefully reviewing the statistical results presented in Table 8, it is found that Egger's test (indicated by the p value of >0.01) provides sufficient evidence to suggest that there is no significant publication bias in the present study. This finding is particularly important as it demonstrates that the study's results were not influenced by any potential biases that may have arisen during the publication process.

4. Detection Techniques of *Brucella* Milk and Dairy Products

4.1. Isolation Techniques of Brucella spp. from Milk and Dairy Products. The isolation method is the most accurate way to diagnose brucellosis [115]. This method is highly specific and allows for biotyping of *Brucella* species isolates [116]. Without particular supplements such as blood, serum, or tissue extracts, *Brucella* species development is weak in simple liquid media [117]. Moreover, without intense movement of the liquid medium, *Brucella* species typically develop poorly [118]. However, it has been proven that *Brucella* species cultures can grow properly on solid media and colonies can be easily identified. *Brucella* species grow well on *Brucella* medium base, tryptone soya agar, glycerol dextrose agar, and sucrose dextrose agar containing 5% bovine or horse serum [117]. It is best to use a nonselective Castaneda's medium to isolate *Brucella* species from milk, blood, or any other bodily fluid. Due to the large number of contaminants present, a selective medium is advised for the primary isolation of *Brucella* species from most clinical specimens [119]. To isolate *B. abortus* from contaminated milk samples, selective media such as Farrell's medium are used, which contain the antibiotics bacitracin, nalidixic acid, vancomycin, nystatin, polymixin B, and cycloheximide [120]. However, Farrell's medium cannot isolate *B. melitensis* because of the growth-inhibitory effects of

Table 5: Reported cases of human brucellosis due to intake of milk and dairy products.

Consumed milk and other products	Geographical areas	Total number of samples	Percentage of human brucellosis patients	Average percentage of human brucellosis patients	Methods of diagnosis	References
Unpasteurized milk	China (2018)	1	100	100	Serum tube agglutination test and culture	[4]
Unpasteurized milk	Ethiopia (2021)	70	33.9	33.9	RBPT, ELISA	[100]
Untreated milk	Horn of Africa (2016)	3	100	100	Culture	[101]
Fresh cheese		469	100		Not determined	[20]
Unpasteurized dairy products		73	100		STA and 2ME	[96]
Local cheese		22	2.2		Biochemical and serological test	[95]
Untreated milk and raw milk products	Iran (2004–2016)	40	100	69.55	STA and 2ME	[96]
Untreated milk		47	58		Standard tube agglutination test (STA) and 2-mercaptoethanol (2ME)	[97]
Untreated milk		Meta-analysis	57.1		Meta-analysis	[102]
Fresh cheese	Israel (2012–2018)	15	100	100	Culture	[21]
Untreated milk and fresh cheese		306	100		Questionnaire method	[22]
Untreated raw milk	Malaysia (2015)	79	87.3 (ELISA)17.5 (PCR)	87.3 (ELISA)17.5 (PCR)	ELISA, PCR	[98]
Untreated raw milk	Oman (2001)	375	63	63	ELISA	[103]
Raw milk	Pakistan (2018)	34	17	17	RBPT, ELISA	[104]
Untreated raw milk	Qatar (2016)	14	100	100	STAT	[105]
Untreated milk	Rwanda (2017)	198	6.1	6.1	Culture	[106]
Untreated milk	Saudi Arabia (2017)	163	45.4	45.4	STAT	[107]
Cheese from goat	Spain (2003–2011)	11	100	100	Culture	[108]
Untreated milk		3	100		RBT	[109]
Cheese from untreated milk		7	100		RBT	[110]
Untreated milk and cheese	Turkey (2003–2010)	283	—	63.6	STAT	[100]
Fresh cheese		1028	63.6		STAT	[99]
Raw milk of cow	USA (2018)	1	100	100		[111]
Raw milk	West Palestine (2021)	1692	37.2	59.05	PCR	[112]
White cheese		3679	80.9			[112]

Table 6: Meta-analysis proportion for the prevalence of brucellosis due to the intake of milk and dairy products.

Studies/References	Geographical areas	Sample sizes	Proportion (%)	95% CI	Weight (%)	
					Fixed	Random
[4]	China (2018)	34	17.647	6.764–34.532	0.42	4.64
[100]	Ethiopia (2021)	70	34.286	23.348–46.600	0.84	4.78
[101]	Horn of Africa (2016)	163	45.399	37.596–53.372	1.95	4.87
[20]	Iran (2004–2016)	469	100.000	99.217–100.000	5.59	4.91
[96]		73	100.000	95.072–100.000	0.88	4.79
[95]		22	2.200	22.726–54.199	0.49	4.68
[96]		40	100.000	42.178–71.742	0.57	4.71
[97]		47	57.447	42.178–71.742	0.57	4.71
[21]	Israel (2012–2018)	15	100.000	78.198–100.000	0.19	4.32
[22]		306	100.000	98.802–100.000	3.65	4.90
[98]	Malaysia (2015)	79	87.342	77.951–93.760	0.95	4.80
[103]	Oman (2021)	375	62.933	57.825–67.836	4.47	4.91
[105]	Qatar (2016)	14	100.000	76.836–100.000	0.18	4.29
[22]	Rwanda (2017)	198	6.061	3.170–10.347	2.37	4.88
[107]	Saudi Arabia (2017)	163	45.399	37.596–53.372	1.95	4.87
[108]	Spain (2003–2011)	11	100.000	71.509–100.000	0.14	4.15
[109]		3	100.000	29.240–100.000	0.048	3.15
[110]		7	100.000	59.038–100.000	0.095	3.85
[100]	Turkey (2003–2010)	1028	63.619	60.593–66.566	12.24	4.92
[111]	USA (2018)	1	100.000	2.500–100.000	0.024	2.32
[112]	West Palestine (2021)	1692	37.175	34.867–39.528	20.13	4.93
[112]		3679	80.892	79.583–82.151	43.76	4.93
Total (fixed effects)		8387	70.180	69.190–71.157	100.00	100.00
Total (random-effects)		8387	73.058	59.433–84.764	100.00	100.00

Table 7: Test for heterogeneity for the prevalence of human brucellosis.

Q	2792.4439
DF	22
Significance level	$P < 0.0001$
I^2 (inconsistency)	99.21%
95% CI for I^2	99.09–99.31

Table 8: Publication bias for the study related to the prevalence of human brucellosis.

Egger's test	
Intercept	−0.3356
95% CI	−7.0674–6.3962
Significance level	$P = 0.9184$
Begg's test	
Kendall's Tau	0.01597
Significance level	$P = 0.9150$

nalidixic acid and vancomycin [119]. To isolate *B. melitensis*, a modified Thayer–Martin medium is used. In order to isolate *Brucella* species, various medium bases are employed, such as Columbia blood agar (Bio Merieux), tryptone soya agar (Oxoid), serum dextrose agar (Oxoid), and *Brucella* medium base (Oxoid). Commercially available selective additives include chocolate agar, BCYE (polymyxin, anisomycin, and cefamandole), and other selective media [117]. Skirrow's agar is used to isolate *B. abortus, B. suis, B. melitensis, B. canis,* and *B. ovis* from infected uterine discharge and milk [121]. Combined with TSA, malachite Brucella medium (MBM) is utilized to recover *B. abortus* RB 51 and rifampin Brucella medium [122].

To maintain milk cleanliness and prevent the spread of organisms, washing and drying the entire udder and teats of the cow before collecting milk samples are recommended. The initial milk flow from all four quarters should be discarded, and 10 ml of midstream milk should be collected to isolate the organism. Extra caution is necessary to prevent contamination from the milker's hand, including proper hand washing, glove use, clean milking equipment, identification and management of sick cows, and adequate training and education for the milker. According to Islam et al. [1], for bacteriological examination of milk samples, it is recommended to refrigerate them at 4°C overnight and then centrifuge them using an Eppendorf tube at 1500 rpm for 15 minutes. The resulting upper portion and sediments should be collected and streaked on *Brucella* selective agar media. After inoculation, the media should be incubated at 37°C with a CO_2 concentration of 5–10% for 7–21 days. During this period, the characteristic morphology, growth rate, and agglutination properties of the colonies should be examined to identify the specific type present. It is important to note that *Brucella* species require a minimum incubation period of 3–5 days [123] to grow on solid media. Certain strains may only produce noticeable colonies on days 14–21 on selective media [118]. Strict biosecurity measures must be followed during manipulations to isolate *Brucella* species. These measures include personnel training, access control, proper PPE, decontamination, appropriate engineering controls, record keeping, emergency procedures, and risk assessment. Differentiation of colonies must be done based on cultural traits, Gram staining [124, 125], growth requirements for serum and carbon dioxide, urease activity, hydrogen sulfide formation, and oxidase tests.

Representative colonies should be preserved for a long time at −80°C in 15% glycerol with TSB or another recommended medium.

4.2. Serological Detection of Brucella-Specific Antibody in Milk. The laboratory diagnosis of brucellosis involves direct and indirect testing methods [126]. Serological testing is an indirect approach used for detecting *Brucella* species in milk. Direct techniques, such as classical bacteriology and PCR or PCR-based methods, are used for identifying *Brucella* species depending on the epidemiological situation in a particular area. While conventional diagnosis relies on serological tests, these tests can lead to false-positive reactions [127]. To detect *Brucella*-specific antibodies in milk, a range of tests are employed, including the standard tube agglutination test (STAT), Rose Bengal plate test (RBPT), 2-mercaptoethanol test (MET), rivanol test, milk ring test (MRT), complement fixation test (CFT), radio immunoassay (RIA), indirect ELISA (iELISA), competitive enzyme immunoassay (cELISA), and fluorescence polarization assay (FPA) [126, 128]. MRT and ELISA are the most widely used methods for identifying *Brucella* antibodies in milk [128]. MRT is commonly used as a screening test in herds, while the milk iELISA test is a precise, sensitive, and cost-effective method for testing many individuals or bulk samples [1, 129]. Potassium chloride extraction of the organism in bulk tank milk samples from dairy herds, followed by ELISA, is a highly specific and reliable method for monitoring brucellosis control programs [129]. Combining ELISA and PCR tests provides 100% sensitivity for detecting *Brucella* antibodies in milk [129]. False-positive reactions are a significant issue when using a serological test, as crossreacting antibodies can cause false positives. The major O-specific polysaccharide (OPS) is the primary source of cross-reactions, and it is almost identical in *Yersinia enterocolitica* O : 9 and *B. abortus*. In addition to these serological cross-reactions, smooth *Brucella* species have been associated with *Francisella tularensis, Salmonella serotypes, Vibrio cholerae, Pseudomonas maltophilia,* and *Yersinia enterocolitica* serotype O : 9 [129]. As the disease progresses to a chronic state, seronegative dairy calves may have decreased antibody titers or undetectable levels of antibodies. Therefore, it is recommended to culture the specimen to confirm that the milk is free of *Brucella* contamination [1].

4.3. Molecular Detection Techniques of Brucella spp. Molecular methods are crucial in diagnosing and implementing control programs for *Brucella* spp. [33, 130, 131]. These methods can provide information about the species and biotype levels of the isolated bacteria and can also distinguish virulent strains from vaccine strains. The type of PCR technique used for diagnosing *Brucella* infections depends on whether the amplification of a particular gene of the genus, species, or biotype is necessary. Furthermore, the choice of the molecular technique is determined by the sample type, whether for diagnosis, molecular genotyping, characterization, or epidemiological surveys. The sensitivity of most molecular techniques ranges from 50% to 100%, and the specificity varies from 60% to 98%. The effectiveness of these techniques depends on the DNA extraction procedure, sample type, and the chosen molecular method [132]. Additionally, a modified technique has been developed to detect *Brucella* species in camel milk, which amplifies the *16S rRNA, omp2*, and *IS711* genes [67]. The qPCR tests, which utilize the *IS711* gene, present a unique and effective approach to detect *Brucella* in milk samples [133]. This gene is a distinctive marker that specifically targets the presence of *Brucella* in the samples, thereby enabling accurate and rapid identification of the pathogen. The application of qPCR-based assays for *Brucella* detection in milk samples has gained significant popularity due to its high sensitivity and specificity and ability to produce results in a timely manner. Thus, using the *IS711* gene in qPCR tests has proven to be a valuable tool for ensuring the safety and quality of dairy products.

Various PCR-based techniques utilize primers developed from different polymorphic areas of the genome for the molecular detection of *Brucella* spp. For example, the B4/B5 primer pairs recognize a 31-kDa protein encoded by a specific gene found in all *Brucella* spp. [134]. The primer pairs P1/P2 and JPF/JPR detect *Omp 2a, Omp 2b*, and *Omp31* as well as the *Omp25/Omp31* of *Brucella* spp [135, 136]. The F4/R2 primer pair is used to amplify *16S rRNA* in *B. abortus* [137]. Multiple multiplex PCR assays have been developed to identify *Brucella* at both the species and biovar levels. AMOS PCR, which uses five primers, allows differentiation among *Brucella* species and is particularly useful for identifying some specific biovars of *Brucella* spp. [138]. Specifically, this method can identify *B. abortus* (1, 2, and 4) biovars [138]. Another multiplex AMOS PCR test has been designed to distinguish the S19 and RB51 *B. abortus* vaccine strains from field strains [139]. An enhanced multiplex PCR assay has also been used to differentiate *B. abortus* biovars 3b, 5, 6, and 9 [130]. The Bruce-ladder multiplex PCR assay is another method that has been developed to recognize and differentiate between all *Brucella* species and vaccine strains [131]. Additionally, a random amplified polymorphic DNA (RAPD) technique is available, a modification of the AMOS PCR. By combining the primers of AMOS PCR with those specific for *IS711*, the resulting RAPD-PCR product can be used for strain typing and differentiation of *Brucella* spp. This PCR assay exhibits high discrimination power and can differentiate all recently recognized *Brucella* spp. [140–143]. An improved method of multiplex PCR assay has been developed to detect all classical *Brucella* spp. This method is particularly useful because it can accurately differentiate between the different classical *Brucella* spp. The assay amplifies specific DNA fragments unique to each *Brucella* spp., allowing for reliable identification. This method builds on the existing techniques for *Brucella* detection, such as AMOS PCR and Bruce-ladder multiplex PCR assay. However, it has the advantage of being able to detect all classical *Brucella* spp., including *B. melitensis, B. abortus*, and *B. suis*. This is important because these species can cause severe illness in animals and humans. The improved multiplex PCR assay is a cost-effective and rapid method for detecting classical *Brucella*

spp. It has the potential to be used in a variety of settings, such as veterinary clinics, diagnostic laboratories, and the field. It is an essential development in the fight against *Brucella* infections and can help to ensure the timely and accurate diagnosis of these infections [144].

Xu et al. [145] recently developed a dual-biosensor approach based on RPA-CRISPR/Cas 12a to detect *Brucella* spp. This technique has a speed and accuracy equivalent to the RT-PCR approach and is powerful in distinguishing the four main *Brucella* species from other bacterial species. It is a valuable tool for the early diagnosis of *Brucella* species in milk. Primer extension techniques have also been developed for single nucleotide polymorphisms (SNPs) that provide a powerful genotyping method to describe the actual phylogenetic framework of *Brucella* spp. All classical *Brucella* species can be rapidly identified using SNPs [146].

Bricker et al. [147] were the first to use multiple locus variable number of tandem repeat analysis (MLVA)-based typing for *Brucella*. Several MLVA tests have since been developed, including MLVA-8, MLVA-11, MLVA-13, MLVA-15, and MLVA-16, for genotyping *Brucella* species and biovars [148]. Vergnaud et al. [149] used MLVA16 on a larger scale to evaluate the genetic diversity of *Brucella* spp. Their study demonstrated the effectiveness of MLVA as a quick and efficient method for determining the genetic diversity of bacterial populations. In a survey of milk and other clinical samples, Islam et al. [28] found that all *B. abortus* Bangladeshi isolates were identical to three Brazilian isolates and one French isolate and very similar to Chinese isolates, according to the findings of the MLVA-16 assay.

5. Preventive Measures to Stop the Transmission of *Brucella* Infection to Humans from Milk and Milk Products

The prevention, control, and eradication programs primarily involve pasteurizing milk and milk products, depopulation, vaccination, and testing and reducing infection reservoirs. Vaccination of cattle is particularly effective in preventing the spread of field strains of the organism. All stakeholders, including livestock producers, veterinarians, field workers, the local community in endemic areas, and regulatory officials, should comprehensively understand these measures [23]. For a program to be successful, it must have well-defined rules and regulations that are followed, and they should be customized to cater to the specific needs of particular regions or herds [128].

5.1. Pasteurization of Milk and Other Dairy Products. Pasteurizing milk is a significant safety measure in areas endemic to brucellosis. Knowing how to avoid consuming unpasteurized milk and milk products is crucial to preventing human brucellosis. The primary goal of pasteurization is to eliminate pathogenic organisms and make milk and other milk products safe for human consumption. However, it is essential to note that pasteurized milk can still contain *Brucella* species if the pasteurization process is not monitored correctly [150]. To ensure the safety of milk and milk products, it is recommended to conduct molecular tests to analyze the presence of *Brucella* in both raw and pasteurized milk [56]. These tests can detect even low bacteria levels and help prevent brucellosis outbreaks. Therefore, it is essential to maintain strict monitoring and quality control measures throughout the milk production process to ensure that the milk and milk products are safe for human consumption [150].

5.2. Surveillance of Brucella Species in Individual and Bulk Milk Using the Milk Ring Test (MRT). The MRT for *Brucella* species surveillance in individual and bulk milk is crucial in controlling brucellosis. The milk ring test (MRT) is widely regarded as the most effective method for detecting brucellosis in animals or potentially contaminated herds. It is a quick, simple, acceptable, affordable, and efficient technique that can significantly aid in the early diagnosis of the disease. The test involves placing a milk sample in a ring or well on a card containing a colored indicator. If the milk sample contains *Brucella*, the bacteria will react with the indicator and cause a visible color change, indicating a positive result for brucellosis. The MRT has been extensively used as a screening tool for detecting brucellosis in dairy herds worldwide, making it an important diagnostic tool in both developed and developing countries [151]. Moreover, the MRT can also be used for monitoring the success of brucellosis control programs. Therefore, it is essential to perform MRTs periodically, especially in areas where brucellosis is endemic. Implementing MRT as a regular surveillance tool makes it possible to detect infected animals early, prevent disease spread, and protect both animal and human health.

5.3. Vaccination Program. Proper vaccination is crucial in preventing brucellosis infection within and between species [152]. Several brucellosis vaccines are available, including S19, RB51, live vectored vaccine, lysate, *B. melitensis* Rev.1, mucosal vaccine subunit, and DNA [153–155]. S19 is a live-attenuated vaccine strain of *B. abortus* and is commonly used to vaccinate young female cattle against brucellosis. RB51 is a live-attenuated vaccine strain of *B. abortus* that is used on both male and female cattle and has proven to be very effective in preventing brucellosis in cattle and some bison herds. A live vectored vaccine employs a benign virus or bacteria to deliver antigens from the target pathogen to provoke an immune response against the targeted pathogen while preventing disease [152]. A lysate vaccine is made by lysing the cells of the target pathogen and using the resulting cellular debris as the vaccine to stimulate an immune response to a broad range of antigens from the target pathogen [152]. *B. melitensis* Rev.1 is a live-attenuated vaccine strain of *B. melitensis* commonly used to vaccinate young female sheep and goats against brucellosis [152]. A mucosal vaccine subunit is a vaccine that is administered to mucosal surfaces and contains only a specific antigen or part of the target pathogen, rather than the whole pathogen, to stimulate an immune response to the target antigen without causing disease [152]. A DNA vaccine triggers an immune response

using genetic material that encodes the target antigen, producing the target antigen by the body's cells, which then triggers an immune response [153–155].

The two most frequently used vaccinations on cattle are RB 51 and *B. abortus* strain 19 [156, 157]. The S19 vaccine produces long-term immunity but is only used on young animals as it can cause abortion in pregnant animals [24]. In contrast, RB51 does not affect the results of serological tests but cannot produce long-term immunity [158–161]. Although a DNA vaccine has been developed that is more effective than S19 and RB51, several booster doses are needed to achieve desirable immunity, similar to RB51 and S19 [162, 163]. Therefore, an effective and safe vaccine is still required to prevent and eradicate brucellosis [23]. Currently, no safe vaccine is available for humans; so, animal vaccination may play a crucial role in managing and eradicating human brucellosis.

5.4. Public Awareness. Public awareness of zoonotic diseases, such as brucellosis, is crucial for preventing their spread and controlling outbreaks. It is essential to educate individuals about the transmission of these diseases, the symptoms, and the measures that can be taken to reduce the risk of infection. Improving public awareness and education can help reduce the incidence of diseases and promote early detection and treatment. Efforts to increase public awareness of brucellosis can include public health campaigns, training programs for healthcare workers, and educational initiatives in schools and communities. Raising awareness and promoting knowledge about brucellosis can help prevent its transmission and improve the health and wellbeing of individuals and communities at risk. According to a review study by Zhang et al. [164], a small percentage of individuals in India (13.7%), Sri Lanka (11.6%), Angola (23.9%), Ethiopia (17.3%), Zimbabwe (21.0%), and Senegal (0.0%) were aware of brucellosis. Overall, the pooled awareness level for brucellosis was 55.5%. Different aspects of brucellosis were examined, revealing awareness levels of 37.6% for the zoonotic nature of the disease, 35.9% for the method of transmission, 41.6% for the symptoms of human brucellosis, and 28.4% for the symptoms of animal brucellosis. Veterinarians and medical workers demonstrated the highest levels of awareness and understanding of human brucellosis, while dairy farmers (15.4%) and abattoir workers (2.6%) had lower levels of awareness. Livestock owners (farmers) had a substantially higher understanding of the zoonotic nature of brucellosis [165]. Additionally, 44.5% of the people knew drinking raw milk posed a risk of contracting brucellosis. The study concluded that Asia and Africa had the least awareness and education regarding brucellosis, emphasizing the need to increase knowledge and awareness about the disease among individuals with links to certain occupations to control the disease effectively. In particular, proper action should be taken to improve public knowledge and awareness about brucellosis in countries with low milk production hygiene standards. Personal protective clothing or equipment and good hygienic measures can prevent occupational exposure to *Brucella* during milking [165]. To reduce the risk of *Brucella* infection in humans, several measures can be effective, including reducing the number of animals in urban areas, limiting human-animal contact, promoting public health awareness campaigns, improving veterinary care, avoiding handling of small ruminants by individuals involved in milking other animals, culling infected animals after screening tests, and isolating infected family members from others [16, 41].

6. Conclusion

In conclusion, human brucellosis caused by consuming raw milk and milk products continues to be a serious public health issue. To address the situation, a comprehensive strategy is necessary that involves creating public awareness about the hazards linked with consuming unpasteurized milk, increasing animal husbandry practices such as vaccination programs and biosecurity measures, enforcing laws governing the sale of raw milk and milk products, and improving surveillance and reporting systems. In order to put into place effective interventions that can lower the prevalence of brucellosis and safeguard human health, it is important to involve all stakeholders, including farmers, veterinarians, medical experts, and policymakers. Together, we can significantly advance the efforts to stop the spread of brucellosis and ensure the safety of our food supply.

Data Availability

Data used to support the findings of this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Md. Sadequl Islam was involved in conceptualizing, planning, evaluating, and interpreting data, authoring the paper, and giving final approval. Md. Ariful Islam participated in the findings' conception, design, and analysis, while Md. Moshiur Rahman contributed to the collection and editing of the data. Dr. Khaleda Islam conducted data evaluation and analysis, and Md. Mominul Islam contributed to the interpretation of the data. Md. Murtuza Kamal summarized the findings, and Md. Nazrul Islam also performed data analysis.

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