



REVIEW ARTICLE OPEN ACCESS

Systematic Review of Vaccine Strategies Against *Tritrichomonas foetus* Infection in Cattle: Insights, Challenges, and Prospects

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ABSTRACT

Tritrichomonas foetus is a protozoan parasite that causes bovine trichomonosis (also referred to as trichomoniasis) resulting in substantial economic loss in extensive grazing systems. The parasite colonises the reproductive tracts of both male and female cattle, being asymptomatic in males but causing early reproductive failure in infected females. This systematic review aimed to examine research manuscripts describing the development of *T. foetus* vaccines, the strategies employed, and the immune response associated with *T. foetus* infection. A systematic review was conducted using indexed sources on Scopus, PubMed, Web of Science, and Embase to search for published vaccine development studies in English utilising either experimental or commercial *T. foetus* vaccines for bovine trichomonosis. Search terms (cattle, bull, heifer, vaccine, immune response, and *Tritrichomonas foetus*) were entered into the indexed sources and with no limit was set for the year of publication. Among 374 studies imported for screening, 96 were duplicates, and 255 were excluded for irrelevancy as these studies did not meet the inclusion criteria (report original data, vaccine tests in cattle, and used either commercial or experimental vaccines that incorporated antigens from *T. foetus*). A further five studies were excluded after full-text review as either the publication described a different target pathogen or the full text was not in English. Eighteen studies met the inclusion criteria and were subjected to data extraction using the Covidence platform. Studies included in the systematic review reported three vaccine strategies, including subunit, cell fraction, and whole-cell killed vaccines. This review considers the design, rationale, and results of each of the 18 studies to provide a comprehensive overview of the current knowledge and to inform future research agendas. While whole-cell killed vaccines are most prevalent, they have shown superior efficacy compared to subunit vaccines studied to date. One study using a purified fractions of *T. foetus* cell membranes as vaccine demonstrated higher efficacy and higher calving rates compared to the whole-cell killed vaccine. All of these methods require cell culture growth of *T. foetus* which can be challenging compared to the production of recombinant proteins. At this stage, no *T. foetus* recombinant antigens have been reported. Advances in understanding the parasite's genome, pathogenesis, host–parasite interactions, and host immune responses to *T. foetus* will provide opportunities for the development of novel vaccine strategies for bovine trichomonosis.

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1 | Introduction

Trichomonas foetus is a flagellated protozoan parasite that infects the bovine reproductive tract causing trichomonosis, resulting in significant reproductive losses [1]. The disease has also been referred to as “trichomoniasis”, however following the recommendations provided by the ‘Standardized Nomenclature of Parasitic Diseases’ (SNOPAD), the term “trichomonosis” is the recommended nomenclature [2]. In addition to reproductive losses, *T. foetus* infection can reduce weaning weights and significantly increase herd culling rates [3, 4]. *Trichomonas foetus* primarily infects and can persist in the male reproductive tract (Figure 1). The preferred site of colonisation of *T. foetus* is the preputial cavity and the penile mucosa, specifically on the surface of the stratified squamous epithelium of the penis and the preputial fornix [5]. Furthermore, the infection may persist for many years in bulls, and the impact and humoral immune response in the lumen of the prepuce are unclear [5, 6]. In the female reproductive tract, the parasite colonises the vagina, cervix, uterus, and oviducts (Figure 1), and although the infection is often self-limiting, some infected females become carriers [7]. The parasite is primarily acquired during breeding [8], resulting in early reproductive failure. One of the significant challenges in managing *T. foetus* infections globally is that there are no drugs registered for treatment in cattle [9, 10]. Most control measures are based on identifying and culling infected bulls since they act as a reservoir of infection [11].

Detection of infected cattle involves either culture or polymerase chain reaction (PCR) testing of reproductive tract samples

[12–15]. Various media have been developed for the transportation and cultivation of *T. foetus*, such as modified Plastring medium [16], Trichomonas medium (Oxoid), modified Diamond’s medium [17, 18] and InPouch TF medium (BioMed Diagnostics). These media support the growth of *T. foetus* differently, with the highest growth rates and cell concentrations observed at different times depending on the medium used [19]. In addition, numerous PCR methods have been reported for detecting *T. foetus* in preputial smegma of bulls or cervicovaginal mucus in cows. For instance, a two-step diagnostic approach using TFR1-2 and TFR3-4 PCR assays targets the 5.8S ribosomal RNA gene and flanking internal transcribed spacer (ITS) regions [20]. Another method, quantitative PCR (qPCR), utilises a 3’ minor groove binder-TaqMan DNA probe targeting the ITS-1, demonstrating significantly higher sensitivity compared to culture followed by microscopy. Additionally, loop-mediated isothermal amplification (LAMP) targeting the 5.8S rDNA subunit has been designed for diagnosing *T. foetus* in smegma cultures, showing slightly higher sensitivity than PCR amplification with TFR3 and TFR4 primers [21, 22].

The parasite’s pathogenesis is attributed to its ability to infect mucosal surfaces of the reproductive tract, bind to spermatozoa, and release a cysteine protease (CP) that induces cell death [23–25]. Although invasion of deeper tissues may occur [26], *T. foetus* predominantly resides on the mucosal surfaces of the host reproductive tract. Studies have shown that *T. foetus* exhibits high cytotoxicity towards human cervical cell lines (HeLa) and an early bovine lymphosarcoma cell line (BL-3) [27] and is capable of killing these host cells by

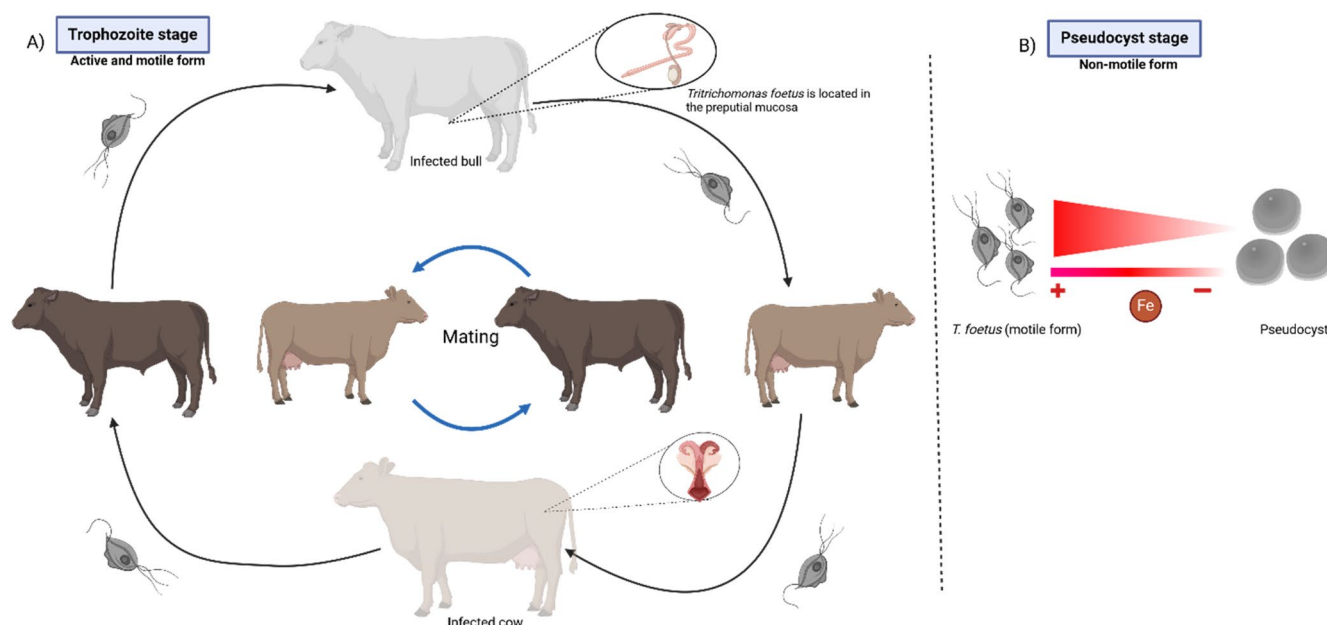


FIGURE 1 | Lifecycle of *Trichomonas foetus* has two stages. (A) Trophozoite stage is the active, motile form of the parasite. Trophozoites are pear-shaped and have flagella that allow them to move. *Trichomonas foetus* is found in the reproductive tract of cattle. The parasite resides in the penile preputial cavity of bulls, specifically on the crypts of the penis and the proximal preputial mucosa in the fornix area. In female cattle, the parasite can colonise the entire genital system, including the vagina, cervix, uterus, and oviduct, within 2 weeks of infection. (B) Pseudocyst Stage. Under certain conditions, trophozoites can transform into pseudocysts. These are non-motile and more resistant to environmental stress. Pseudocysts of *T. foetus* form under specific stress conditions, particularly when there is a depletion of essential nutrients. One key factor is the depletion of iron from the environment. When iron is scarce, the trophozoites transform into pseudocysts to survive the nutritional stress. Additionally, cooling and warming schemes can also induce pseudocyst formation and reversal.

contact-dependent activity [27, 28]. Consequently, adhesion of *T. foetus* to host cells might be an important step in cytotoxic damage of these targets and may thus be regarded as an essential factor in pathogenesis. Molecules localised on the parasite surface as well as on the surface of the host cells are involved in the adherence of *T. foetus* [25]. A monoclonal antibody reacting with a 190-kDa *T. foetus* surface molecule complex, termed adhesin Tf190, was shown to inhibit adhesion of *T. foetus* to HeLa cells and impede parasite cytotoxicity in vitro [28]. This suggests an important role for Tf190 in the adhesion and killing of host cells in vivo.

Trichostrongylus axei adheres to cells in the reproductive tract, including epithelial cells in the vagina, uterus, and oviduct, and trophoblast cells in the developing placenta of the pregnant cow [29]. Adherent *T. foetus* cells release cysteine protease 30 (CP30), which causes cytopathic effects and apoptosis of the infected cells [30–32]. The release of CP30 also leads to the cleavage of IgG2, allowing the parasite to evade the host's immune response [30, 32]. The adherence of *T. foetus* to spermatozoa results in a loss of motility, agglutination, and the release of spermicidal lysozymes [31, 33]. Persistently infected bulls, which act as asymptomatic carriers, transmit the parasite to cows during mating [34] (Figure 1). In addition to natural mating, mechanical transmission can occur through contaminated artificial insemination (AI) equipment or if contaminated instruments are used for the gynaecological examination of cows [35]. Furthermore, semen from infected bulls used for AI can passively transmit *T. foetus* present in the urethra and preputial cavity.

Bovine trichomonosis is a global disease, and although it has been eradicated from some regions due to enhanced screening combined with AI, it remains a significant concern in countries that use extensive grazing systems and natural mating [8]. To control the disease, some countries have implemented programmes to identify and remove infected bulls using microscopic examination of samples derived from preputial scraping or washes and confirmation through PCR or LAMP [12, 13, 15, 21, 22]. Testing and culling infected bulls [36] and providing at least 3 months of sexual rest for infected females [8, 37] have been reported to be effective control measures. The culling of old bulls is particularly effective as they are more likely to harbour *T. foetus* compared to younger bulls, as the organism establishes within the microscopic folds on the penis and the lining of the prepuce [38, 39]. Furthermore, older bulls tend to have more wrinkled penises [40], leading to multiple folds that create an increased formation of crypts. These crypts serve as an ideal microenvironment to facilitate the growth of facultative or microaerophilic anaerobic microorganisms [5].

Currently, there are no approved treatments for cattle infected with *T. foetus*; however, there is some anecdotal evidence that the vaccine can be used as a therapeutic [34, 41]. While some nitroimidazole drugs have shown promise as drug treatments [42, 43], none are approved for use in cattle and other food-producing animals. In dogs and cats, metronidazole offers a treatment option for giardiasis [44], but metronidazole's effectiveness is restricted to certain strains of *Giardia*,

and it can induce adverse effects [44]. Consequently, while 5'-nitroimidazoles, including metronidazole, are routinely used to treat protozoal infections in humans, their use in veterinary medicine remains limited and due to reported adverse reactions such as carcinogenicity and teratogenicity in laboratory mice after prolonged exposure [45]. Therefore, the use of metronidazole has been banned in food-producing animals such as cattle [45]. An attractive approach to preventing bovine trichomonosis is vaccination. Two commercial vaccines, Tricovac (Argentina) [46] and TrichGuard (USA) [47], have been developed using whole-cell killed *T. foetus* cells mixed with special oil adjuvant (proprietary) and are recommended for subcutaneous vaccination of female cattle. Additionally, similar experimental vaccines based on whole-cell killed *T. foetus* using different inactivation approaches (lyophilisation, formalin, and formaldehyde) [46, 48, 49] and adjuvants such as Quil-A, water in oil, oil in water, and mineral oil, and alternative delivery methods, that is, vaginal administration were developed [48, 50]. These experimental vaccines have shown promise in reducing the number of infected cows, shortening the period of genital infection, and increasing the pregnancy rate compared to unvaccinated animals [46, 48–50]. In the early 1980s, Australian researchers developed a vaccine for bulls similar to TrichGuard [41]. This vaccine was developed with killed *T. foetus* protozoa sourced locally to produce optimal efficacy but was never commercialised as the prevalence of bovine trichomonosis in Australia was thought to be insignificant [41]. While experimental whole-cell killed vaccines have shown promise in reducing the severity of *T. foetus* infections, their effectiveness in preventing the colonisation of the genital tract warrants further investigation. However, recent advancements, such as the development of Trichobovis [48], have demonstrated significant progress in clearing the infection and improving conception rates. Previously, a comprehensive analysis of the effectiveness of whole-cell killed *T. foetus* vaccines in beef cattle was done [51]. These findings highlight the ongoing advancements in research and development towards more effective vaccines.

Other experimental vaccines have also been developed that contain subunits or cellular fractions of *T. foetus*, such as fractionated cells [52–57]. These vaccines have shown the potential to increase systemic antibody levels and reduce the duration of infection in cows. Studies suggest that subunit vaccines for *T. foetus* in cattle have the potential to induce protective immune responses and clear the infection [52, 53, 58]. However, developing subunit vaccines for *T. foetus* presents several challenges, including identifying suitable antigens, effective adjuvants to enhance the immune response, and rigorous testing to ensure safety and efficacy. Despite these challenges, the potential benefits of such vaccines make them a worthwhile research focus in the ongoing effort to control *T. foetus* infections in cattle.

This systematic review evaluates the current state of vaccine development against *T. foetus* and identifies gaps and future research directions. Specifically, it summarises different vaccine types, immune responses, and challenges associated with vaccine development. By systematically examining the available literature, this review aims to provide a comprehensive

overview of the current state of vaccine development against bovine trichomonosis and to inform future research efforts in the field.

2 | Materials and Methods

2.1 | Research Selection and Search Criteria

To collate the available research articles published reporting vaccine development against bovine trichomonosis, a systematic approach was adopted, and searches were conducted in four electronic databases: PubMed, Web of Science, Embase, and Scopus. This systematic review was framed around the review title “bovine trichomonosis vaccine development” by using the keywords “cattle”, “bull”, “heifer”, “vaccine”, “immune response”, “trichomonas foetus”, “t. foetus”, and “trichomonas foetus” for the searches. The detailed search strategies for each database are listed in Supporting Information S1. The literature search was conducted on 4th April 2024 with no temporal restriction to ensure more comprehensive data and to include maximum research studies in this regard. The results generated by the databases were imported to EndNote, and duplicates were removed.

All research articles published in peer-reviewed journals were included in the review. We excluded the results that did not report original data, including reviews, correspondence, conference abstracts, and book chapters. The research articles were downloaded, and articles that did not have full English text available were also excluded. The title and abstracts of all records were screened by two independent reviewers to filter out studies that performed vaccine tests in hosts other than cattle. In the selection of studies for inclusion, emphasis was placed on investigations employing vaccines whether commercially available or experimental that incorporated antigens from *T. foetus* and the target animals were bovine. Exclusion criteria included studies that utilised antigens not originating from *T. foetus* or were directed towards non-bovine species (Figure 2).

2.2 | Data Extraction

Studies that met our inclusion criteria were subjected to data extraction using the Covidence platform to obtain the following information: journal name, year of publication, vaccine type, adjuvants used, gender and age of cattle, vaccination regimen, dosage, safety, immunogenicity, efficacy of the vaccine, challenge route, and dose. Covidence is a web-based collaboration software platform that streamlines the production of systematic and other literature reviews (Covidence systematic review software 2024, Veritas Health Innovation, Melbourne, Australia, available at www.covidence.org). Covidence can facilitate the screening and filtering of studies based on predefined criteria such as keywords, authors, and publication year(s). The platform supports structured data extraction, ensuring consistency and reducing omissions. Additionally, Covidence also promotes collaboration among multiple reviewers to assess the risk of bias in studies. Integration with reference management software such as EndNote and Zotero can further streamline the management of references. These features collectively enhance the efficiency, reliability, and reproducibility of our systematic review.

3 | Results

3.1 | Studies Included in the Systematic Review

In total, 374 studies were retrieved from the customised searches. After removing duplicates, the first screening was undertaken with 278 studies. After the first screening, 255 studies were removed, including records that did not report original data, records that did not have full English text, and those that studied animals other than cattle. Full texts of the remaining studies ($n=23$) were further screened for eligibility, and 18 studies were eventually subjected to data extraction (Figure 2). Thus, this systematic review identified 18 vaccine studies for *T. foetus*. In these 18 papers, 14 focused on *T. foetus* prophylactic vaccines (Tables 1 and 2) and four studies utilised the vaccine as a therapeutic (Table 3).

The eighteen *T. foetus* vaccine papers were published between 1983 and 2022. Figure 3A shows the number of papers published. Geographically, the studies reviewed were mainly from the USA ($n=10$), Argentina ($n=4$), Australia ($n=2$), South Africa ($n=1$), and Spain ($n=1$) (Figure 3B). Figure 3C depicts the number of papers stratified by type of vaccine (whole-cell killed, cellular fractions, and subunit), indicating that whole-cell killed ($n=12$) vaccines was the most frequently used vaccine strategy, comprising 67% of the reviewed papers. In summary, Figure 3A suggests that research in *T. foetus* vaccine development is expanding, and the 2000s up to 2017–2022 were the most prolific in terms of publications. Geographically, the USA is at the forefront of this research, and whole-cell killed vaccines are the most studied vaccine type.

3.2 | Characteristics of *T. foetus* Vaccines

A total of 12 studies reported the development of whole-cell killed vaccines [46–50, 54, 57, 59–63], two studies developed a vaccine from cell fractions [50, 57], and four studies focused on the development of subunit vaccines [52–54, 58] as their vaccine strategy against *T. foetus* (Figure 3C, Table 2). Tables 1 and 2 summarise the outputs of these publications by describing the type of vaccine, sex, age, commercial or experimental vaccine used, inactivation method, adjuvant, vaccination regimen, dosage, safety, challenge method and dose, efficacy, and immunogenicity. Fourteen of the vaccine studies focused on vaccinating females with the vaccine used as a prophylaxis (Tables 1 and 2), and four studies used males for therapeutic vaccination (Table 3). For the inactivation of the cultured protozoa for the vaccine, three studies utilised lyophilisation [47, 48, 60], five used formaldehyde [46, 49, 50, 59, 61], and two used formalin [41, 57].

In this systematic review of 18 *T. foetus* vaccine studies, we have provided a comprehensive summary of different types of vaccines, specifically focusing on whole-cell killed, subunit, and cellular fraction vaccines (Tables 1 and 2), and Table 3 shows therapeutic vaccination in bulls. The data presented in the efficacy column of Tables 1 and 2 provide a summary of the effectiveness of these *T. foetus* vaccines. Most of the studies (8/18) included in this review that utilised whole-cell killed vaccines have demonstrated a shortened duration of *T. foetus* infections in cows [46, 48–50, 59, 61, 62] and improve pregnancy and calving rates [47]. A study compared the efficacy of whole-cell killed *T. foetus* vaccine to cellular fractions and found that whole-cell

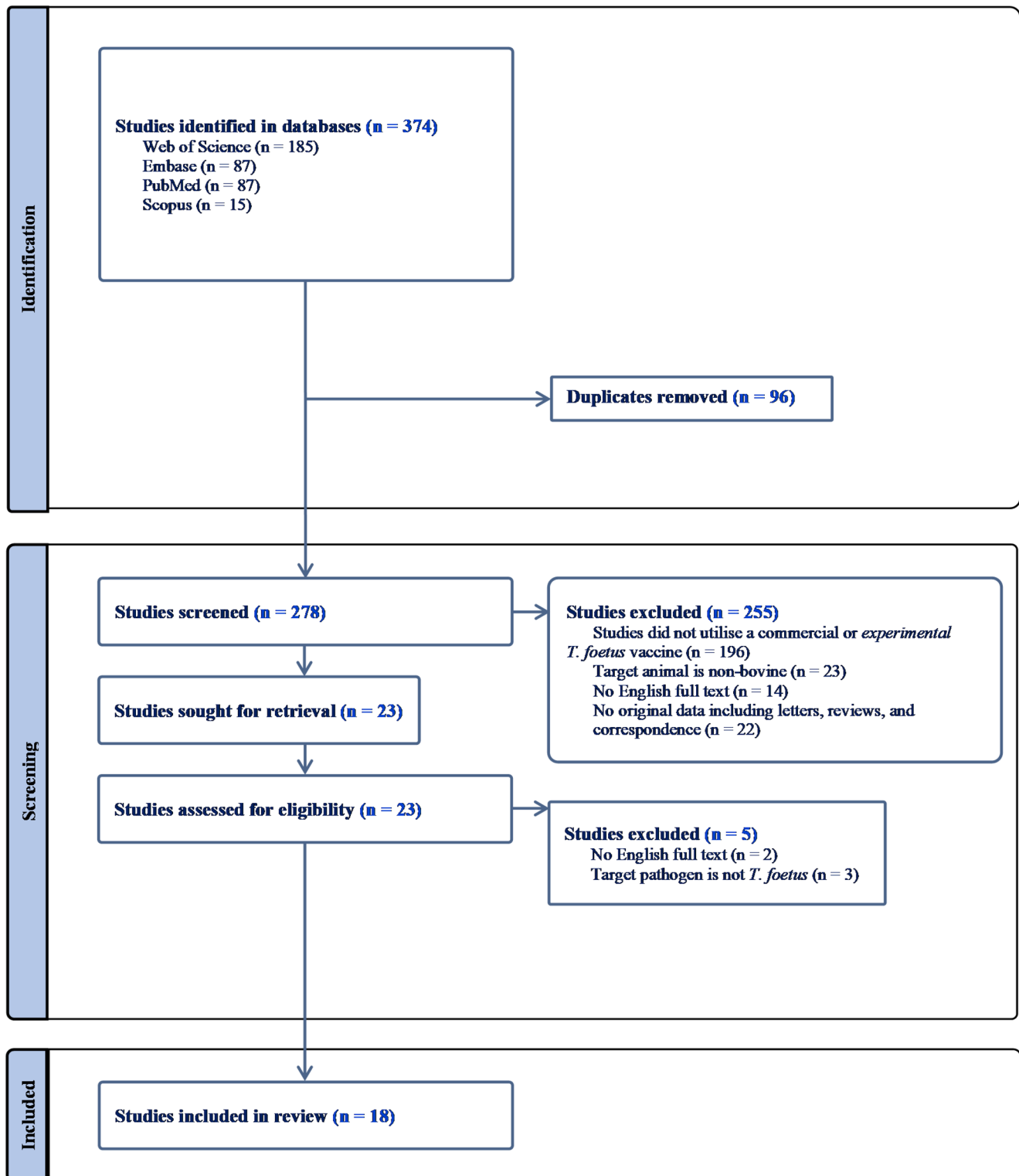


FIGURE 2 | Flow chart of the study selection and identification process. The steps were adopted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

killed vaccines showed better efficacy compared to cellular fractions for the reduction of *T. foetus* infections in cows [50]. Subunit and cellular fraction vaccines, which contain only specific proteins or cellular fractions of *T. foetus*, also play a crucial role in preventing bovine trichomonosis. Subunit vaccine studies included in this review (2/14) have shown that immunisation with TF1.17 surface antigen [52, 58] from *T. foetus* can result

in faster parasite clearance and reduced infection rates in cows. Vaccines from cellular fractions of *T. foetus*, such as membrane glycoproteins, have demonstrated that heifers immunised using these fractions had lower mean persistence of genital infection, higher pregnancy rates, and lower reproductive losses, with significant differences compared to unvaccinated females [50, 57]. It is challenging to definitively recommend one type of *T. foetus*

TABLE 1 | Commercial vaccines used against *T. foetus* infection in cattle.

Sex and age	Commercial				Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
	Vaccine type	vaccine used	Inactivation method	Adjuvant					
Female, 24–36 months old	Whole-cell killed	TrichGuard		Special oil adjuvant (proprietary)	Two SC injections followed by an SC boost. Immunisations were given 3 weeks apart.	Artificial challenge: IV via inoculation of motile 2×10^6 trophozoites ($> 99\%$ viability) of a bovine isolate of <i>T. foetus</i> (isolate no. 14) into the fornix using a Cassou pipette. Natural challenge: Cattle were exposed to infection with <i>T. foetus</i> by natural mating with the naturally infected bulls of the herd throughout a 120-day mating period.	The average number of days of positive <i>T. foetus</i> isolation was shorter in the vaccinated group using TrichGuard (36.65 ± 25.92 days) compared to the non-vaccinated/challenged group (60.12 ± 32.57 days).	Stimulated high serum anti- <i>T. foetus</i> IgG and genital IgA levels and generated an IgG booster effect like TrichGuard	[48]
Female, 12–14 months old	Whole-cell killed	TrichGuard	Lyophilisation	Special oil adjuvant (proprietary)	Two SC injections given 2 weeks apart.	Artificial challenge: Heifers were IV inoculated during the synchronised estrus with 2×10^6 organisms of a cloned isolate of <i>T. foetus</i> of bovine origin (CDTf-4).	Vaccine significantly increased pregnancy and calving rates and reduced losses associated with <i>T. foetus</i> infections.	Not discussed	[47]

(Continues)

TABLE 1 | (Continued)

Sex and age	Vaccine type	Commercial vaccine used	Inactivation method	Adjuvant	Vaccination regimen	Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
Female, 15–18 months old	Whole-cell killed	Tricovac	Not discussed	Oil adjuvant	Two SC injections followed by intravaginal boost. Immunisations were performed with 20 days interval.	5 mL dose contains 50% oil adjuvant and 50% suspension of 5×10^7 <i>T. foetus</i> , 6655 strain. Adjuvant inflammation ^b	Artificial challenge: Challenge was carried out by using a Cassou pipette to deliver 2 mL of PBS containing 1.5×10^6 <i>T. foetus</i> B1 motile cells/mL (> 99% viability) into the fornix.	Median time for clearance of infection was 69 days in non-immunised animals, 55 days in animals treated with aluminium hydroxide, 41 days with oil-in-water or saponin based vaccines or with a commercial vaccine and 27 days in animals treated with saponin plus aluminium hydroxide.	Vaccinated animals IgG levels increased after the first vaccine application regardless the used adjuvant. Oil-in-water and saponin based vaccines showed abrupt slopes in IgG concentration and reach a plateau after 2 weeks.	[46]
Female, 12–14 months old	Whole-cell killed	TrichGuard	Lyophilisation	Special oil adjuvant (proprietary)	Three SC injections. Immunisations were given 2 weeks apart.	2 mL dose SC. A second dose administered 2 weeks later. ^c	Natural challenge: Cows were with infected Angus bulls in the same pasture for natural mating within with a 3-month breeding season.	Not discussed	Vaccination of beef cows with a commercial vaccine containing killed <i>T. foetus</i> induced significant increase in the levels of IgG antibodies.	[60]

Abbreviations: IM, intramuscular; IN, intranasal; IV, intravaginal; SC, subcutaneous.

^aSafety not discussed.

^bAnimals vaccinated with the Montanide ISA 206 VG which exhibited granulomatous inflammation the disappeared around day 60 of the trial.

^cNo adverse side effects.

TABLE 2 | Experimental vaccines used against *T. foetus* infection in cattle.

Sex & age	Vaccine type	Experimental vaccine used	Inactivation method	Adjuvant	Vaccination regimen	Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
Female, 24–36 months old	Whole-cell killed	Experimental: Isolate no. 96 of <i>T. foetus</i> , which was obtained from the smegma of a Spanish infected bull [66].	Lyophilisation	Quil-A	Two SC injections followed by an SC boost. Immunisations were given 3 weeks apart.	2 mL per dose containing 5×10^7 lyophilised trophozoites, 0.4 µL of the protease inhibitor and 750 µg of Quil-A adjuvant. ^a	Artificial challenge: IV via inoculation of motile 2×10^6 trophozoites ($> 99\%$ viability) of a bovine isolate of <i>T. foetus</i> (isolate no. 14) into the fornix using a Cassou pipette. Natural challenge: Cattle were exposed to infection with <i>T. foetus</i> by natural mating with the naturally infected bulls of the herd throughout a 120-day mating period.	The average number of days of positive <i>T. foetus</i> isolation was shorter in the vaccinated group using Trichobovis (28 ± 10.76 days) compared to the non-vaccinated/challenged group (60.12 ± 32.57 days).	Stimulated high serum anti- <i>T. foetus</i> IgG and genital IgA levels and generated an IgG booster effect like TrichGuard	[48]
Female, 18–24 months old	Whole-cell killed	Experimental: <i>T. foetus</i> B1 strain obtained from an isolate from a cow with pyometra [48, 57].	Formaldehyde	Not discussed	Single intravaginal immunisation	1 mL dose of 2×10^8 cells/mL in 0.5% formaldehyde. ^b	Artificial challenge: Infection consisted of instillation of 1 mL of PBS containing 10^6 <i>T. foetus</i> B1 strain ($> 99\%$ viability) IV using a Cassou pipette.	The median time for parasite clearance among unvaccinated heifers was 93.75 days while in immunised animals was 45 days.	Formaldehyde fixed <i>T. foetus</i> did not stimulate cervical IgA.	[49]
Female, 15–18 months old	Whole-cell killed	Experimental: The strain B1 of <i>T. foetus</i> originally isolated from a cow with pyometra [57].	Formaldehyde	Several adjuvants were used ^c	Two SC injections followed by intravaginal boost. Immunisations were performed with 20 days interval.	Vaccine dose (5 mL) contained 10^8 cells/mL of <i>T. foetus</i> . Adjuvant inflammation ^d	Artificial challenge: Challenge was carried out by using a Cassou pipette to deliver 2 mL of PBS containing 1.5×10^6 <i>T. foetus</i> B1 motile cells/mL ($> 99\%$ viability) into the fornix.	Median time for clearance of infection was 69 days in non-immunised animals, 55 days in animals treated with aluminium hydroxide, 41 days with oil-in-water or saponin based vaccines or with a commercial vaccine and 27 days in animals treated with saponin plus aluminium hydroxide.	Vaccinated animals IgG levels increased after the first vaccine application regardless the used adjuvant. Oil-in-water and saponin based vaccines showed abrupt slopes in IgG concentration and reach a plateau after 2 weeks.	[46]

(Continues)

TABLE 2 | (Continued)

Sex & age	Vaccine type	Experimental vaccine used	Inactivation method	Adjuvant	Vaccination regimen	Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
Female, 18–24 months old	Whole-cell killed	Experimental: cells of <i>T. foetus</i> and <i>C. fetus</i> subspecies <i>venerealis</i>	Formaldehyde	Oleo adjuvant consisting of Marcol 52, Arlcel C, and Tween 80	Two SC injections followed by IV boost. Immunisations were performed at monthly intervals.	8 mL dose containing 1.62×10^8 of <i>T. foetus</i> , 16 mg of <i>C. fetus</i> subsp. <i>venerealis</i> , and 8 mg of <i>C. fetus</i> subsp. <i>venerealis</i> ^b <i>intermedius</i> .	Natural challenge: All heifers were exposed to Infection with <i>T. foetus</i> and <i>C. fetus venerealis</i> by mating them with two naturally infected bulls over a 90-day breeding period. Artificial challenge: Received an inoculum via IV of 1×10^8 live <i>C. fetus venerealis</i> cells and 4×10^6 live <i>T. foetus</i> cells.	Systemic priming and vaginal boosting with an experimental dual vaccine applied Prior to natural or natural and supplemental challenge resulted in faster clearance of <i>T. foetus</i> and <i>C. fetus</i> infection before what is considered to be a critical point (70 days after conception and infection).	Heifers immunised with the experimental vaccine had a faster rise in systemic antibodies during and after a 90-day breeding period.	[61]
Female, 12 months old	Whole-cell killed	Experimental: <i>Campylobacter fetus</i> , <i>Leptospira canicola-hardjo-icterohaemorrhagiae-pomona bacterin</i> with killed <i>T. foetus</i> .	Not discussed	Not discussed	Two SC injections given 3 weeks apart.	5 mL dose containing 5×10^7 killed <i>T. foetus</i> . ^d	Natural challenge: Heifers in the natural challenge-exposure (NC) group were infected by breeding to <i>T. foetus</i> -infected bulls. Artificial challenge infected: Heifers in the experimental challenge-exposure (EC) group were infected by IV with 10 mL of growth medium containing 10^7 <i>T. foetus</i> .	Mean duration of infection in unvaccinated heifers was 10.2 weeks compared to 3.2 and 3.5 weeks for heifers of the natural challenge-exposure (NC) and experimental challenge-exposure (EC) groups, respectively.	Predominant trichomonal antibody isotype in serum was IgG1. Trichomonal IgA and IgM antibodies also increased.	[62]
Female, 18–24 months old	Whole-cell killed	Experimental: Culture of preputial wash specimen from an infected bull (isolate and source not mentioned).	Not discussed	Water in oil adjuvant	Two IM injections given 6 weeks apart.	5 mL dose containing 1×10^7 <i>T. foetus</i> . Only a single heifer exhibited swelling at the injection site.	Natural challenge: Heifers were infected by breeding to a <i>T. foetus</i> infected bull.	The duration of the infection in the unvaccinated heifers averaged 14 weeks as opposed to 7 weeks in the vaccinated.	Not discussed	[63]

(Continues)

TABLE 2 | (Continued)

Sex & age	Vaccine type	Experimental vaccine used	Inactivation method	Adjuvant	Vaccination regimen	Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
Male, 36–96 months old	Whole-cell killed	Experimental: Cells of <i>T. foetus</i> var. Brisbane	Formalin	Mineral oil adjuvant	Three SC injections given monthly	10 mL dose containing 10 mg dry weight <i>T. foetus</i> cells/mL. ^b	Natural challenge: Bulls were exposed to infection with <i>T. foetus</i> var. Brisbane by mating them with infected cows.	<i>Tritrichomonas foetus</i> challenge resulted in permanent infection of 2 of 8 (25%) vaccinated, and 7 of 8 (87.5%) unvaccinated bulls.	One month after the third injection of vaccine, titres of serum agglutinins against <i>T. foetus</i> ranged from 160 to 640 (arithmetic mean 320) in vaccinated and from 0 to 10 in control bulls.	[41]
Male, 24–36 months old	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	2 of 21 (9.5%) vaccinated and 8 of 19 (42.1%) unvaccinated bulls became permanently infected.	Four weeks after the third injection of vaccine, titres of serum agglutinins against <i>T. foetus</i> ranged from 40 to 320 (arithmetic mean 80) in vaccinated, and from 0 to 10 in control bulls.	[41]
Female, 12 months old	Whole-cell killed	Experimental: Inactivated <i>T. foetus</i> (strain/source not mentioned).	Formaldehyde	Mineral oil adjuvant	Two SC doses of the vaccine given 3 weeks apart.	1 × 10 ⁹ <i>T. foetus</i> cells in 2 mL of adjuvant. ^b	Natural challenge: Heifers were infected by mating them with <i>T. foetus</i> infected bulls.	Fewer vaccinated heifers became infected than unvaccinated controls (69.4 vs. 93.0%, respectively) and vaccinated heifers tend to clear the infections earlier than the controls (48.9 vs. 68.5 days, respectively).	Not discussed	[59]

(Continues)

TABLE 2 | (Continued)

Sex & age	Vaccine type	Experimental vaccine used	Inactivation method	Adjuvant	Vaccination regimen	Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
Female, <48 months old	Whole-cell killed	Experimental: Inactivated <i>T. foetus</i> from Colorado State University's Veterinary Diagnostic Laboratory from a bull.	Formaldehyde	Mineral oil adjuvant	One SC injection of inactivated vs. two SC boosts of inactivated given 3 weeks apart.	1 mL dose containing 1×10^9 <i>T. foetus</i> . ^b	Artificial challenge: Bulls were infected by infusing with 2.5×10^5 <i>T. foetus</i> organisms in 1 mL of culture medium into the prepuce with an insemination pipette.	Inactivated vaccine was superior to fragmented cell vaccines, and vaccinating twice was superior to one vaccination for the elimination of trichomonad infections in dairy cows.	No significant increase in serum titres for <i>T. foetus</i> was detected anytime in either unvaccinated or vaccinated groups.	[50]
Same as above	Cellular fractions	Fractionated cells using flow sonicator—same strain as above	Not applicable	Same as above	Same as above	1 mL dose containing 1×10^9 <i>T. foetus</i> or equivalent protein per dose (exact dose not mentioned). ^b	Same as above	Same as above	Same as above	[50]
Female, 18–24 months old	Whole-cell killed	Experimental: Inactivated vaccine of <i>T. foetus</i> B1 strain was prepared according to Clark, Duffy [41].	Formalin	Mineral oil adjuvant	Two SC doses followed by one IV dose injections given at monthly intervals.	5 mL dose containing 1×10^8 cells. ^a	Natural challenge: Mating with <i>T. foetus</i> positive bulls and to further ensure infectivity, the bull was infused into the preputial cavity with $1.5\text{--}2 \times 10^6$ <i>T. foetus</i> cells fortnightly during breeding season.	Heifers immunised using the cellular fraction vaccine had lower mean persistence of genital infection, higher pregnancy indexes, and less reproductive losses, with significant differences with regard to non-vaccinated females and the whole cell vaccine.	Cellular fraction vaccine using <i>T. foetus</i> membrane induced a better systemic immune response during service compared to the inactivated vaccine.	[57]
Same as above	Cellular fractions	Vaccine was prepared according to Clark, Emery [34].	Not applicable	Same as above	Same as above	6 mL dose containing 300 µg of membrane proteins. ^a	Same as above	Same as above	Same as above	[57]

(Continues)

TABLE 2 | (Continued)

Sex & age	Vaccine type	Experimental vaccine used	Inactivation method	Adjuvant	Vaccination regimen	Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
Female, 12–14 months old	Subunit	Experimental: Tf190 adhesin antigen from a Montana strain of <i>T. foetus</i> originally isolated in 1985	Not applicable	Aluminium hydroxide	Two SC injections followed by an IN boost. Immunisations were given 1 month apart.	100 µg/mL of Tf190 antigen	Artificial challenge: Cows were infected IV with 10 ⁶ live <i>T. foetus</i> strain TF330.1	Immunisation with the purified adhesin, Tf190, decreased the infection rate after challenge (66% in controls versus 33% in immunised animals).	Systemic immunisation with Tf190 results in serum antibody production and antiparasitic adhesion antibodies.	[53]
Same as above	Same as above	Same as above	Same as above	Same as above	One SC injection and two IN boost given 1 month apart.	100 µg/mL (SC dose) and 100 µg Tf190 plus 20 µg cholera toxin subunit B (IN dose)	Same as above	IN immunisation of cattle resulted in a 50% decrease in cumulative infection rate in Tf190-immunised animals (33% infection rate) compared to that in controls (66% infection rate).	Evidence of systemic immunisation was absent in animals immunised IN with Tf190, and antigen-specific antibody responses in cervical mucus samples from these animals were undetectable 75 days after the initial immunisation.	[53]
Female, 12–14 months old	Subunit	Experimental: Tf190 adhesin antigen from a Montana strain of <i>T. foetus</i> originally isolated in 1985.	Not applicable	Aluminium hydroxide	Three SC injections. Immunisations were given 2 months apart.	100 µg/mL of Tf190 antigen. ^b	Not challenged	Whether primed CD4+ T-cells that produce IFN-γ lead to a protective response against IV <i>T. foetus</i> challenge has yet to be determined.	CD4+ T cells increased in Tf190-primed PBMCs after antigen re-exposure suggesting Tf190-specific CD4+ T cells could be an important memory T-cell phenotype that respond to antigen by expression of IFN-γ.	[54]
Same as above	Whole-cell killed	Experimental: Irradiated whole cells	Ultraviolet	Same as above	Same as above	500 µg of protein per dose in aluminium hydroxide. ^b	Not challenged	Not discussed	Not discussed	[54]

(Continues)

TABLE 2 | (Continued)

Sex & age	Vaccine type	Experimental vaccine used	Inactivation method	Adjuvant	Vaccination regimen	Dosage and safety	Challenge method and dose	Efficacy	Immunogenicity	Study
Female, 12–24 months old	Subunit	Experimental: Surface antigen (TF1.17) of <i>T. foetus</i> strain D1 originally obtained from a cow with pyometra [67].	Not applicable	IFA adjuvant	Three IM injections given 3 weeks apart.	2 mL dose containing 100 µg of TF1.17 of <i>T. foetus</i> . ^b	Artificial challenge: Heifers were infected with 10 ⁶ live <i>T. foetus</i> (D1 isolate) in 1 mL of PBS inoculated IV of each heifer.	75% of the vaccinated heifers cleared the parasite by week 6 of the trial compared to 10 weeks in unvaccinated heifers.	Systemic immunisation with immunoaffinity purified TF1.17 surface antigen stimulated serum IgG1 and IgG2 antibody response.	[52]
Female, 12–14 months old	Subunit	Experimental: Immunoaffinity-purified superficial antigen (TF1.17) of <i>T. foetus</i> strain D1 obtained from a cow with pyometra [68]	Not applicable	Incomplete Freund's adjuvant (IFA) or dextran sulphate plus IFA.	Three IM injections given 3 weeks apart.	100 µg of TF1.17 antigen. ^b	Artificial challenge: Heifers were challenged with 10 ⁶ <i>T. foetus</i> strain D1 inoculated IV.	Vaccination with purified superficial antigen TF 1.17 not only resulted in faster clearance of the challenge organisms than in control, but also showed that animals that cleared infection faster had less histologic evidence of endometritis than those animals clearing later.	IgA antibody responses to the immunoaffinity-purified superficial antigen, <i>T. foetus</i> strain D1 were detected by 6 weeks in infected animals with increases in mean response at 8 and 10 weeks.	[58]

Abbreviations: IM, intramuscular; IN, intranasal; IV, intravaginal; SC, subcutaneous.

^aNo adverse side effects.

^bSafety not discussed.

^cQuil-A, Aluminium hydroxide, Quil-A + aluminium hydroxide, and Montanide ISA 206 VG.

^dAnimals vaccinated with the Montanide ISA 206 VG which exhibited granulomatous inflammation the disappeared around day 60 of the trial.

TABLE 3 | Therapeutic vaccine studies for *T. foetus* infection in cattle.

Sex and age	Vaccine type	Commercial/experimental vaccine used	Inactivation method	Adjuvant	Therapeutic regimen	Dosage and safety	Efficacy	Study
Male, 48–72 months old	Whole-cell killed	Commercial: TrichGuard	Lyophilisation	Special oil adjuvant (proprietary)	Two SC injections given 2 weeks apart.	2 mL dose SC followed by a booster 2 weeks later. ^a	Therapeutic immunisation of bulls did not produce improvement in infection clearance relative to the control group (9/10) despite robust humoral response to vaccination.	[66]
Male, 36–60 months old	Whole-cell killed	Experimental: Culture of preputial wash specimen from an infected bull (isolate and source not mentioned).	Not discussed	Water in oil adjuvant	Two IM injections given 6 weeks apart.	5 mL dose containing 1×10^7 <i>T. foetus</i> . ^a	Therapeutic immunisation of 110 infected bulls resulted with 69/110 bulls still positive after treatment.	[63]
Male, 48–108 months old	Whole-cell killed	Experimental: Cells of <i>T. foetus</i> var. Brisbane	Formalin	Mineral oil adjuvant	Three SC injections given monthly.	10 mL dose containing 10 mg dry weight <i>T. foetus</i> cells/mL. ^a	Therapeutic immunisation resulted with 11 of the 16 (68.8%) vaccinated and 1 of the 8 (12.5%) unvaccinated bulls became free of infection.	[41]
Male, 60–72 months old	Cellular fractions	Experimental: Glycoprotein or membranes of <i>T. foetus</i> var. Brisbane [41]	Not applicable	Mineral oil adjuvant	Three SC injections given monthly.	5 mL dose containing 500 µg of membrane proteins or 160 µg of glycoprotein. ^a	Therapeutic immunisation of four infected bulls resulted with 3/4 bulls negative after treatment, immunised bulls. This suggests that infected bulls can be therapeutically immunised against trichomonosis with vaccines containing either the membranes or the membrane glycoproteins of <i>T. foetus</i> .	[34]

Abbreviations: IM, intramuscular; SC, subcutaneous.

^aSafety not discussed.

vaccine over another due to the limited number of studies that have directly compared the whole-cell killed, cellular fractions, and/or subunit vaccines. While the data from this review suggest that the clearance time for subunit vaccines is longer than any whole-cell killed vaccine, and one single fraction vaccine study showed the best clearance rates compared to whole-cell killed and higher calving rates [50].

Two different challenge methods were used (Figure 3D) to assess their impact on vaccine efficacy. One method involved natural challenge through mating with an infected animal usually heifers with *T. foetus*-infected bulls [48, 57, 61–63]. Another method was experimental, involving intravaginal inoculation of live *T. foetus* cells at specific concentrations ranging from 1×10^2 to 1×10^7 cells [62]. Parasite challenge was either conducted using one of these approaches or both. Two studies that utilised both challenge approaches in their vaccine studies [61, 62] demonstrated a slight difference in terms of vaccine efficacy (lower rate of *T. foetus* infection) naturally challenged vaccinated heifers. For instance, the number of vaginal mucus samples that were negative for *T. foetus* and *Campylobacter fetus* subspecies *venerealis* was statistically higher in vaccinated heifers that were naturally challenged (6/10 samples) compared to artificially challenged (4/11 samples) [61]. Given the limited number of studies on *T. foetus* vaccines that directly compare and analyse vaccine efficacy between naturally and artificially challenged heifers, it is difficult to definitively confirm the best approach. Further research is needed to elucidate this aspect.

Up to 2002, the most common adjuvant used in experimental vaccines was mineral oil [41, 59, 61, 63], followed by aluminium hydroxide [53, 54]. More recently, a comparison of four adjuvants used with the whole-cell killed *T. foetus* vaccine demonstrated that Quil A mixed with aluminium hydroxide led to the fastest clearance of *T. foetus* infection (27 days), followed by Montanide ISA 206 VG or Quil A (41 days) with aluminium hydroxide clearing at 55 days [46]. Two or three doses of whole-cell killed vaccines every 3 to 4 weeks are the most common vaccination regimens used. For the commercial vaccine TrichGuard (Boehringer Ingelheim, USA), the adjuvant used by the company is proprietary and not reported on Safety Data Sheets. However, for Tricovac (Biotandil, Argentina), the adjuvant is as Marcol-52 oil-based adjuvant: Montanide ISA 50 V2 (Seppic, France).

3.3 | Effectiveness of Multi-Component Vaccines

Vaccines comprising whole cells have been demonstrated to confer protection in cows and are commercially available [64]. These vaccines can be either monovalent or part of a polyvalent formulation, containing *Campylobacter*, *T. foetus*, and *Leptospira* spp. [8]. Evidence suggests that these vaccine formulations exhibit effectiveness in cows, but their efficacy in bulls remains unproven [65]. A systemic vaccination approach using whole-cell killed of *C. fetus* subspecies *venerealis* and *T. foetus* has been trialled [61]. This dual vaccine was administered to cows that were mated with bulls infected with both pathogens for a duration of 90 days, along with an additional vaginal instillation of both pathogens on day 39 [61]. The vaccine was given subcutaneously, leading to increased systemic and vaginal IgG responses in the vaccinated cattle, reduced infection periods for

each pathogen (*C. fetus* subspecies *venerealis* and *T. foetus*), and a higher pregnancy rate compared to the unvaccinated group [61]. This vaccination method, combining systemic subcutaneous and mucosal vaginal doses before and during the mating period, could have triggered an extended immune response. This study concluded that the regimen covered the crucial risk period in a 2- to 3-month mating program, starting in the later part of the mating season and continuing until a month, which is when most pregnancy losses are observed [61].

3.4 | Vaccination as a Therapeutic

The commercially available vaccine, TrichGuard, has been used, but it cannot prevent *T. foetus* infection entirely, as demonstrated in a therapeutic vaccine trial in which 9/10 bulls were still positive 18 weeks post-treatment [66]. These findings cast uncertainty on the practical advantages of therapeutic immunisation of bulls infected with *T. foetus*. Additionally, studies done by Clark et al. assessed the therapeutic use of whole-cell killed *T. foetus* vaccine and cellular fractions in infected bulls but also failed to confer protection [34, 41]. Lastly, a large therapeutic vaccine trial was conducted with 110 infected bulls and resulted in 69 positive animals post-treatment [63].

3.5 | Immune Responses Against *T. Foetus* Immunisation

A systematic review of 18 vaccine studies on *T. foetus* revealed patterns of immune responses in cattle following immunisations using either *T. foetus* antigens or whole-cell killed vaccines (Tables 1 and 2). Collectively, these studies indicate that elements of both the adaptive and innate immune responses play a role in the recognition of the pathogen, with particular emphasis on complement proteins, antibody responses (IgG and IgA), and CD4+ T-cells. Antibodies and complement proteins can coat pathogens, marking them for attack through opsonisation [67]. Antibodies can also activate the complement system and innate immune cells, such as polymorphonuclear cells and macrophages [68]. The antibody response is driven by B cells and CD4+ T-cells, generating antigen memory.

Most of the studies (12/18) included in this review emphasised serum components, specifically IgG and IgA antibodies. Heifers vaccinated with TF1.17 antigen (an adhesion protein) demonstrated that antigen-specific systemic IgG1 and vaginal and uterine IgG1 and IgA accelerated clearance of *T. foetus* infection before manifestations of lesions or reproductive failure [52, 58, 69, 70]. This vaccine induced serum IgG1 antibody against 50–70 kDa soluble glycosylated antigen (SGA) and 150–200 kDa proteins identified as glycosylated surface lipophosphoglycan (LPG) antigen. This is similar to previously reported reactivity of antibodies to TF1.17 and Tf190 LPG/protein adhesins [71–73]. Tf190 antigen can induce antigen-specific CD4+ T-cell responses resulting in upregulation of the inflammatory cytokine, IFN- γ [53, 54]. This suggests that immunisation with Tf190 adhesin antigen primes T-cell responses in cattle and can lead to IFN- γ mediated effector functions that are antigen-specific. Immunisation also results in increased IgG1 and IgG2 serum antibody production and antiparasitic adhesin antibodies

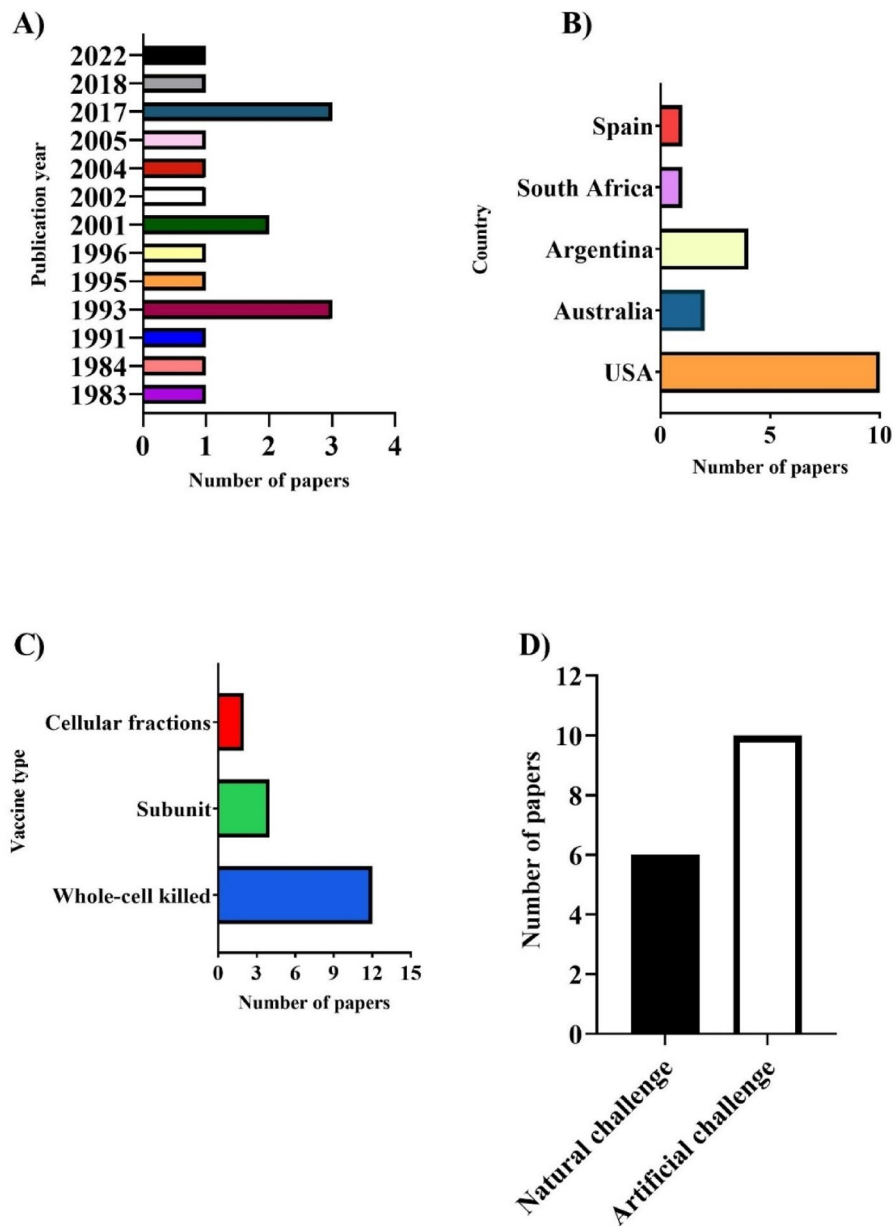


FIGURE 3 | Number of *Trichostrongylus axei* vaccine studies categorised into (A) publication year, (B) country, (C) type of vaccine, (D) challenge method. Naturally challenged animals are those that were mated with *T. axei* infected animal and artificially challenged animals are those that were inoculated with live *T. axei* cells.

[53, 54]. Although these antigens demonstrate stimulation of immune responses, evidence for faster vaginal *T. axei* clearance is either lacking or less successful than whole-cell killed *T. axei* vaccines.

A *T. axei* trophozoite vaccine formulation inactivated by lyophilisation and Quil-A-adjuvanted, as used in Trichobovis, induces promising immune responses [48]. This vaccine stimulated high serum anti-*T. axei* IgG and genital IgA levels and generated an IgG booster effect similar to TrichGuard [48]. The IgA levels were associated with significantly earlier genital clearance of *T. axei* in cows immunised with Trichobovis by the subcutaneous route [48]. The roles of IgG and IgA antibodies are thus crucial as they mediate key immune responses against *T. axei* challenge in vaccinated heifers. These findings suggest that both *T. axei* antigens and whole-cell killed *T. axei* can

elicit specific immune responses in cattle, potentially offering protection against this parasite, as seen in 12 studies that measured higher parasite clearance in the female genital tract after *T. axei* challenge [46–50, 52, 53, 57–59, 61, 62].

4 | Discussion

With the absence of available treatments, vaccination plays a vital role in the control program of bovine trichomonosis, and culling infected cattle is both costly and challenging. The primary aim of vaccines against bovine trichomonosis is to clear *T. axei* infection from the reproductive system of cows, to prevent the establishment of infection, and to prevent embryonic and foetal loss. In the case of bovine trichomonosis, managing the infection primarily involves identifying and

removing infected animals through diagnostic testing and culling, respectively. Our systematic review of 18 vaccine studies against *T. foetus* found that whole-cell killed vaccines have primarily been developed more often than fractionated cell or subunit vaccines. A systematic review and meta-analysis of inactivated vaccines in beef cattle was done previously [51]; however, further studies using whole-cell killed vaccines have been added since 2013, and this current review is comprehensive, including other approaches to vaccine development (subunit and fractionated cell vaccines). Conducting a meta-analysis was not feasible since very few studies compared vaccine types in the same study, and it was not possible to compare vaccine types between papers due to different experimental designs and outcome measures.

In a recent study conducted by Ortega-Mora et al. a novel whole protozoal vaccine formulation (Trichobovis) demonstrated higher efficacy in reducing genital infection time in heifers compared to the commercial vaccine (TrichGuard) [48]. This vaccine formulation was developed using *T. foetus* trophozoites that were inactivated via lyophilisation and adjuvanted with Quil-A. The vaccine effectively elicited a specific immune response that inhibited trichomonad adherence to bovine vaginal epithelial cells [48]. Bovine serum antibodies to whole-cell *T. foetus* antigens have been shown to immobilise and agglutinate trophozoites, enhancing bovine complement and neutrophil-mediated killing of *T. foetus* [29]. Additionally, a recent study explored the interaction between bovine neutrophils and *T. foetus*, focusing on the mechanism of trogocytosis as a means of parasite clearance. This study found that bovine neutrophils effectively kill *T. foetus* through trogocytosis, where the neutrophils surround and ingest parasite fragments, leading to parasite death [74]. The findings suggest that understanding these immune mechanisms could aid in developing more effective vaccines or treatments for trichomonosis in cattle.

Five studies that utilised either experimental or commercial vaccines were based on whole-cell killed *T. foetus* inactivated using chemicals such as formaldehyde or formalin [46, 49, 50, 59, 61]. However, it is recommended to replace these products as they can interact and modify immunogenic epitopes and be potentially toxic and carcinogenic [49, 70, 75]. Lyophilisation used in Trichobovis is a non-hazardous and cost-effective method to inactivate *T. foetus* trophozoites while preserving the parasite proteins [48]. The choice of adjuvant is also important, with Quil-A, a widely used immunostimulatory saponin, most commonly used in livestock vaccines developed in the 1970s [76]. Also, the route of administration of the vaccine may be a critical factor for improving *T. foetus* vaccine efficacy. A single intravaginal immunisation of formalin-fixed *T. foetus* cells significantly shortened *T. foetus* infection time compared to non-vaccinated animals (45 vs. 93.75 days) [49]. In contrast, other studies using two systemic doses of formalin-fixed parasites and a third dose delivered intravaginally did not demonstrate that mucosal delivery improved the overall vaccine efficacy [46, 61, 70].

Although these whole-cell killed vaccines can be successful, there are limitations in the propagation of *T. foetus* cultures to drive mass production and distribution of *T. foetus* vaccines. It would be more effective to produce a recombinant vaccine,

which can be produced through service providers, in comparison to a specialised microaerophilic whole-cell culture vaccine. Fractionated cell vaccines pose those same challenges, yet research to identify the active components of these fractions has not been reported. If fractions are prepared by sonication of *T. foetus* cultures, the whole-cell killed vaccine performs better [50]; however, when membrane extracts are purified using sonication and sucrose gradient purification, the vaccine is more effective than the whole inactivated vaccine, as demonstrated by improved calving rates [57]. Only two types of subunit vaccines have been described specifically: surface antigen TF1.17 and adhesin TF190 [52, 53, 55, 58]. Three studies in our systematic review reported a shorter duration of genital infection when using TF190 or TF1.17 and a higher pregnancy rate in females [52, 53, 55]; however, the clearance time at 6 weeks is much longer than described for whole-cell killed vaccines and the pure membrane fractions [58]. This is likely why these vaccines have not been studied further or commercialised.

In four studies, whole-cell killed and membrane fraction vaccines were used as therapeutic vaccines in *T. foetus*-positive bulls [34, 41, 63, 66]. Initially, Clark et al. suggested that the membrane fraction vaccine was successful as a therapeutic in bulls under 5.5 years of age; however, this was later proven to be flawed by other researchers [34, 41]. For example, in a South African study, 69 out of 110 bulls were still positive after using an experimental inactivated *T. foetus* vaccination [63]. Similar conclusions were made by Alling et al. in 2018 when using TrichGuard, who found that the vaccination of *T. foetus*-positive bulls did not improve the rate of clearance compared to unvaccinated bulls [66]. The 2013 meta-analysis of whole-cell killed *T. foetus* vaccines by Balzell et al. highlighted inconsistencies in the study by Clark et al., citing significant imprecision due to the small sample size (a single study with 12 animals per group) [41, 51]. It also pointed out a high likelihood of publication bias. Furthermore, it noted the peculiarity of the fact that these findings have not been replicated since their initial publication in 1983.

Bovine trichomonosis is known for causing significant inflammatory reactions in the host, which might also lead to immunopathology [58]. However, the detailed immunological processes involved at the mucosal reproductive tract interface have not been thoroughly studied. Another crucial factor in the disease's development that has yet to be explored is the interaction between *T. foetus* and the existing bovine vaginal microbiome. In human infections with *Trichomonas vaginalis*, the parasite has been shown to negatively affect the *Lactobacillus* populations in the vagina, leading to a significant increase in pH levels [77]. Studies on the pathogenic mechanisms of *T. foetus* have mainly focused on identifying and characterising molecules that help the parasite adhere to the host's epithelial cells, such as adhesin Tf190, which has been shown to prevent *T. foetus* from adhering to HeLa cells and reduce parasite toxicity in vitro [28, 72]. Other studies include enzymatic activities that cause tissue damage, such as CPs. These proteinases can effectively cleave fibronectin, an extracellular matrix glycoprotein that mediates cell adhesion, in vitro [78].

Antibody-mediated responses serve as a vital defensive mechanism that the bovine host employs to eliminate *T. foetus*. Whether the antibodies measured actually correlate with protection has

been investigated in studies included in this review, which demonstrated that high anti-*T. foetus* IgG and genital IgA led to a shorter duration of infection compared to unvaccinated animals [46, 48, 49, 52, 53, 58, 62]. Vaccine-induced genital antibodies may have eradicated *T. foetus* from the preputial cavity through immune mechanisms such as inhibiting trichomonad adherence to the mucosa and activating complement and neutrophils [79, 80]. The role of IgG antibodies is further supported by in vitro evidence showing that bovine serum antibodies to whole-cell antigens of *T. foetus* can immobilise and agglutinate *T. foetus*, inhibit its adherence to bovine vaginal epithelial cells, and enhance complement and neutrophil-mediated killing of the parasite [29, 68, 81, 82]. Additionally, antibodies targeting surface LPG and the surface antigen TF1.17 have been associated with genital protection in female cattle [52, 69, 70]. However, studies on bovine trichomonosis have identified mechanisms employed by *T. foetus* to evade these immune responses mounted by the host [30, 32]. Bovine IgG exhibits a nonspecific affinity for the outer membrane of *T. foetus*, and this mechanism has been postulated to camouflage the parasite's surface antigens, thereby preventing immune recognition by the host [53]. This may lead to a delayed inflammatory response, which could be linked to the asymptomatic nature of the infection [83]. *Tritrichomonas foetus* uses membrane-bound and secreted CPs to enzymatically cleave IgG1 and IgG2, thereby hindering the antibody-mediated immune response [30]. However, the bovine host can still eliminate the infection, potentially owing to the resistance of bovine IgA and IgM against *T. foetus* CP cleavage [84]. An additional strategy used by *T. foetus* to evade immune recognition and subsequent clearance through antibody-mediated responses involves the shedding of surface antigens. This shedding hampers the host's ability to identify the parasite, leading to immune evasion. *Tritrichomonas foetus* also undergoes antigenic variation, exemplified by the adhesion complex TF190 present on its surface, which expresses diverse epitopes that impede antibody-mediated clearance [72].

Furthermore, the surface antigen TF1.17, which has been shown to stimulate immune responses and treatment of bovine trichomonosis with monoclonal antibodies directed against the TF1.15-TF1.17 significantly reduced adhesion of bovine *T. foetus* to bovine vaginal epithelial cells [81, 85]. Research has demonstrated that the surface antigen TF1.15-TF1.17 is involved in establishing infection and inducing clinical signs [71]. In addition, the conservation of the TF1.15-TF1.17 antigen among different isolates of *T. foetus* has been studied using TF1.15 and TF1.17 monoclonal antibodies, showing that most isolates express whole antigen recognised by both monoclonal antibodies [73]. However, it was found that some isolates lack the epitope that is recognised by the TF1.17 monoclonal antibody, yet still elicit an immune response to the conserved regions of TF1.15-TF1.17 [73]. All isolates were recognised by the TF1.15 monoclonal antibody suggesting the corresponding region in TF1.15-TF1.17 is conserved. Vaccination studies utilising the surface antigen TF1.15-TF1.17 have demonstrated efficacy in inducing targeted IgG1 and IgA antibody responses in vaginal and uterine secretions [58, 60]. This surface antigen has shown potential in protecting female cattle against *T. foetus* when challenged approximately 7 weeks after vaccination [60] however, this is much longer than whole-cell killed or purified membrane extracts as described above.

Notably, a striking disparity exists between the response to immunisation observed in cows and bulls [86, 87]. Systemic immunisation using *T. foetus* antigens has been shown to reduce the duration of infection and decrease reproductive failures in cows [35, 57, 69, 88]. This type of vaccination primarily triggers an IgG antibody response in both the serum and genital secretions [69, 70, 87]. When a *T. foetus* mucosal booster is administered systemically (either intranasally or intravaginally), it induces the production of IgA antibodies in the vagina and both IgA and IgG antibodies in the uterus [69, 70]. IgA plays a crucial role in impeding the adhesion of invading microorganisms to the mucosal cell wall, thereby preventing further colonisation [89, 90]. The protection conferred to cows following systemic vaccination is largely attributed to IgG antibodies, similar to bulls. However, despite this local immune response in cows, the protective effect is transient, as reinfection typically occurs within 6 to 12 months post-initial infection. The precise reason for the short-lived nature of this protective response remains elusive [63], but it could be due to the lack of CD4+ T memory cells. This highlights the lack of studies focusing on the T-cell response to *T. foetus* infection and is an area where more research is needed. While there are no reports of systemic priming with local boosting in bulls, it is possible that their responses could mirror the vaginal responses observed in cows.

Nonetheless, if the underlying mechanism could be elucidated and the duration of the immune response prolonged accordingly, it could pave the way to develop a vaccination regimen that provides the most effective, long-lasting protection against *T. foetus*. This could involve factors such as the timing of vaccine doses, the type of vaccine used, and the way the vaccine is administered. In contrast to cows, old bulls cannot clear the infection [8]. As bulls age, the mucosa of the penis and prepuce folds changes, leading to notably deeper epithelial crypts compared to those in yearlings or 2-year-olds [8]. This allows *T. foetus* to avoid detection by hiding within these crypts in the male reproductive tract [8]. Consequently, the sole recourse is to cull the infected bull to prevent further spread of the infection [8, 90]. Enhancing our understanding of this host-pathogen interaction and deciphering the countermeasures employed by the host's immune system could contribute to developing novel vaccines for bovine trichomonosis.

4.1 | Prospects

The efficacy of several experimental whole-cell killed vaccines and purified *T. foetus* membrane extracts demonstrates that *T. foetus* clearance rates are shorter compared to unvaccinated heifers. Subunit vaccines are less effective, and recombinant vaccines have not been developed. Although different versions of fractionated cell extracts have been described, *T. foetus* purified membrane extracts have demonstrated slightly better *T. foetus* clearance rates and calving rates compared to whole-cell killed vaccines [34, 57]. *Tritrichomonas foetus* is a flagellated protozoan that exhibits a complex morphological and physiological differentiation process during its lifecycle, transitioning between motile and reproductive stages within the host [91]. In the motile stage, *T. foetus* uses its flagella for movement and to attach to the host's epithelial cells, and this stage is characterised by a pear-shaped body and the presence of three anterior flagella

and one posterior flagellum [92]. The reproductive stage, on the other hand, is when the parasite multiplies through binary fission, and during this stage, *T. foetus* becomes rounder, and the flagella are retracted [93]. Although the lifecycle of *T. foetus* may not exhibit the same level of complexity as some other parasites, it still presents significant obstacles for vaccine development. The lifecycle of *T. foetus* includes two main stages: the trophozoite and the pseudocyst. The surface proteins can vary between these two forms, with certain immunogenic proteins being expressed differently or not at all in each stage. For instance, the adhesin Tf190 is expressed in both trophozoite and pseudocyst forms, but the expression levels and surface localisation differ [93]. Iron depletion has been shown to induce the formation of pseudocysts from trophozoites, accompanied by changes in surface protein expression, which can affect the parasite's ability to adhere to host cells and evade the immune system [94]. This variation in protein expression complicates the identification of consistent antigen targets for vaccine development. Antigenic variation in *T. foetus* has been proposed as a significant mechanism for immune evasion [64]. This hypothesis is supported by observed antigenic variability in the protective surface antigen TF1.17 [52, 73]. Additionally, studies using monoclonal antibodies have shown variation in the expression of epitopes within the TF190 adhesion complex [72]. However, another study did not find detectable differences in the total protein composition [65]. Therefore, it remains unclear whether antigenic variation plays a crucial role in immune evasion during bovine *T. foetus* infection.

4.1.1 | Omics Technologies

Application of omics technologies can be instrumental in the development of vaccines for pathogens such as *T. foetus*. These technologies facilitate the identification of potential antigens within *T. foetus*, which could serve as targets for vaccine development [94]. Alonso et al. conducted a comparative transcriptomic analysis of *T. foetus* which showed an abundance of predicted virulence factors, including proteases and surface antigens [95]. Proteomic analysis could also be used to identify and characterise proteins that are expressed by *T. foetus*, providing insights into the parasite's biology and pathogenicity [95, 96]. A study utilised proteomics to investigate the presence of the 20S proteasome, a protein complex involved in selective protein degradation in *T. foetus* [96]. This proteasome plays a role during the cell cycle and transformation into the endoflagellar form [96]. The characterisation of the 20S proteasome in *T. foetus* was achieved through a combination of whole genome sequencing technologies, bioinformatics algorithms, cell fractionation, biochemistry, and microscopy approaches [96]. Moreover, proteomic analysis can reveal the abundance levels of predicted virulence factors, such as proteases and surface antigens [95]. These factors could be involved in bovine infection and could serve as potential targets for vaccine development.

Lastly, genomics plays a crucial role in the development of vaccines for pathogens, including *T. foetus*. The advent of genomics has revolutionised the field of vaccinology and therapeutic development, transitioning from traditional microbiological approaches to sequence-based strategies. Genome sequencing facilitates the identification of genetic signatures associated with

the virulence of *T. foetus* and genetic factors that contribute to immunity or successful vaccine response. There is an opportunity to utilise genomics and reverse vaccinology approaches to identify novel vaccine candidates [97]. The two currently available *T. foetus* genome assemblies for Belfast and K strains contain 2776 and 3730 contigs, respectively, and vary in size dramatically at 147.0 Mb (Belfast strain) and 67.6 Mb (K strain) [98]. Current third-generation genome sequencing methods [99] could be utilised to develop better genome assemblies, improving whole gene annotations, which could lead to the selection of novel *T. foetus* vaccine candidates. Repetitive genome regions hinder accurate assembly and the generation of a complete and high-quality genome, particularly when using short-read sequencing methods. However, Oxford Nanopore Technology (ONT) and Pacific Biosciences (PacBio) long-read sequencing protocols can resolve these repetitive regions to generate more comprehensive genome assemblies, including structural variants [100, 101]. Lastly, rapid advances in sequencing technologies for sequencing single long DNA and RNA molecules have led to substantial improvements in accuracy, read length, and low error rates [101].

Currently, proteomics, transcriptomics, and metabolomics techniques are key technologies for characterising pathogens and identifying the components associated with pathogen-to-host interactions. Future research should focus on identifying specific proteins that could serve as host immune biomarkers or targets for vaccines. Further investigation into the biological roles of *T. foetus* membrane proteins could provide insights into the mechanisms of pathogenicity and host-parasite interactions. Functional assays could be designed to explore how these proteins contribute to the survival and virulence of *T. foetus*. Combining proteomic data with transcriptomic and metabolomic analyses could provide a more comprehensive understanding of the biological processes in *T. foetus* and how they adapt to different host environments. Also, of potential importance in bovine trichomonosis pathogenicity is the interaction of *T. foetus* with the host vaginal microbiome and whether this can influence trichomonosis infection.

4.1.2 | Systems Vaccinology

Systems vaccinology integrates high-throughput technologies described above with immunology to provide a comprehensive understanding of the immune responses elicited by a vaccine [102]. This approach could be particularly beneficial in the context of vaccine development against bovine trichomonosis. For example, in the case of *T. foetus*, systems vaccinology could help identify key antigens that elicit protective immune responses in cattle. This can include the prediction of antigen secretion, membrane association, and immune binding epitopes (B cell and T cell) using bioinformatic tools. Following this, in silico modelling and machine learning algorithms can be employed to predict the most effective antigen combinations. These predictions must be validated using in vitro and in vivo studies, assessing the immunogenicity and protective efficacy of the candidate vaccines. Finally, systems biology approaches could be utilised to analyse the complex interactions between the host immune system and the parasite, providing insights into the mechanisms of protection and guiding the optimisation of *T. foetus* vaccine

formulation. Thus, the optimisation of *T. foetus* vaccine delivery systems can then be based on developing a protective immune response through the selection of the most appropriate adjuvant and delivery route. As noted in this review, there is an opportunity to use more modern adjuvants, for example, Quil-A and emulsions, as well as delivery systems, for example, microparticles [103, 104] and liposomes [105, 106], in future *T. foetus* vaccine formulations. This integrative strategy not only accelerates the vaccine development process but also enhances the likelihood of developing a safe recombinant vaccine against *T. foetus* in cattle.

4.1.3 | CRISPR/Cas9

CRISPR/Cas9 gene-editing technology has revolutionised the field of molecular biology [107] and holds significant promise for the development of novel vaccines. One potential application of CRISPR/Cas9 for *T. foetus* vaccine development is the targeted disruption of specific genes to attenuate its virulence, thereby creating a live attenuated vaccine [108, 109]. This approach could potentially elicit a robust immune response without causing disease, as the immune system would be exposed to a broad array of antigens. Moreover, CRISPR-Cas9 has been used to make alterations to the genome of *Toxoplasma gondii* by switching off essential genes responsible for the formation of oocysts, which are the infectious stages of this parasite [110].

Also, CRISPR technology has been used extensively in T-cell engineering, particularly in the context of human cancer therapy [111–113]. While specific studies on bovine immune response modulation are not readily available, the principles of T-cell engineering using CRISPR are likely to be similar across species. This technology could potentially be applied to modulate the bovine immune response to *T. foetus* infections by engineering bovine T-cells to kill *T. foetus*; however, further research would be needed to determine the feasibility of such an approach. Lastly, while the potential of CRISPR/Cas9 for vaccine development is immense, there are several challenges that need to be addressed. These include the potential off-target effects of gene editing and the ethical and regulatory considerations associated with genetically modified organisms in the food chain [114, 115].

5 | Conclusions

Trichostrongylus axei infections in cattle adversely affect productivity worldwide, causing significant economic losses. Conventional management practices such as controlled mating and testing followed by culling of the infected animals are variably effective in controlling this venereal infection. Vaccines have shown the potential to reduce the number of infected cows, shorten the period of genital infection, and reduce pregnancy loss. However, the practical advantage of vaccination for *T. foetus* has been questioned owing to the lack of demonstrated long-term immunity. Despite the progress made in *T. foetus* vaccine development, there are still research gaps regarding the efficacy of inactivated vaccines, the optimal adjuvant, the duration of protection, and the prevention of colonisation of the genital tract.

Author Contributions

John Harvey M. Santos: data curation, analysis, visualisation, and writing. **Gry B. Boe-Hansen:** supervision, review, and editing. **Hannah V. Siddle:** supervision, review, and editing. **Ali Raza:** supervision and editing. **Loan T. Nguyen:** supervision and editing. **Kieren D. McCosker:** review and editing. **Michael McGowan:** review and editing. **Ala E. Tabor:** supervision, method validation, review, writing, and editing.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.