


## Review Article

# Bovine Trichomonosis in Cattle: Trends in diagnostic approaches - Challenges and Opportunities

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**Abstract**—Bovine Trichomonosis is a most neglected venereal diseases of cattle that causes severe reproductive failure. With current understanding, early disease detection and keeping diseased animals in confinement are the only viable strategies. There have also been several reports of zoonotic transmission in immunocompromised humans. According to World Organization of Animal Health recommendations, few culture and RT-PCR techniques for detecting *Trichomonas foetus* parasites in clinical samples have been established. Testing could not be expanded to regular screening and point-of-care settings due to a scarcity of testing kits, greater prices, and longer turnaround times. This article provides the trends in development of various diagnostic methods and their applicability. Furthermore this review highlights the limitations of the current methods and proposes the development of easy to use serological testing devices like Indirect ELISA (iELISA) and Lateral Flow Assay (LFA) rapid kit as Point of care testing (POCT) assays that are suitable for effective implementation of screening and monitoring of disease progress in herds.

**Keywords**— Venereal diseases, Culture methods, Point – of – care testing, ELISA, Lateral flow assay

## 1. Introduction

*Trichomonas foetus* (*T.foetus*) was mostly found to colonize the urogenital tract of cattle resulting in reproductive failure. The *T.foetus* was first reported in cattle in 1999 [1] and eventually, it was also verified as the causative organism of chronic diarrhea among domestic cats (*Feline T.foetus*) [2]. *Trichomonas suis* (*T. suis*), a similar pathogen to *T.foetus* was also detected was currently regarded as a commensal among the pigs [3]. However, later studies have demonstrated that it was found to be causing Atrophic rhinitis in pigs [4]. *Trichomonas vaginalis*, a member of the same species that affects around 150 million people worldwide and was once thought to be the most common non-viral venereal disease, is one of the most researched human parasites causing sexually transmitted illnesses [5].

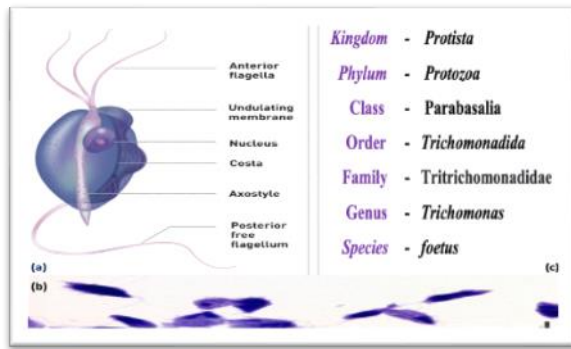
## 2. Morphology

Organisms in the *Trichomonadidae* family are distinguished by the number of flagellae. Thus, *Trichomonas* (*Tritrichomonas*) is distinguished by three flagellae, whereas *Tetratrichomonas* and *Pentatrichomonas* have four and five flagellae respectively. Among the *Tritrichomonadidae* family, only *Tritrichomonas* was considered to be a pathogenic

organism [2,3]. The *T.foetus* isolated from bovine and feline (cat) samples were reported to be morphologically identical, however [6] reported a lack of relationship in its prevalence between cats and cattle. Later [7] clarified the existence of molecular divergence in several discrete gene loci. As proposed in Reference [8] the spillover of feline *T. foetus* into the bovine reproductive system. Further, *T.foetus* has shown similarity at morphological and molecular levels with *Trichomonas suis*, a commensal parasite of pigs.

*T.foetus* is often found as a trophozoite with a pyriform appearance throughout its life cycle. It measures about 8-18µm in length and 4-8 µm in width. It has three anterior flagellae, a posterior flagellum and an undulating membrane as shown along with the phylogenetic status in Fig.1.

With the help of flagellae, *T.foetus* trophozoites were found to move away from aerobic conditions and accumulate in the microaerophilic environment. As discussed in [9] *T.foetus* reside in the skin folds of the vagina and penile regions in cattle.

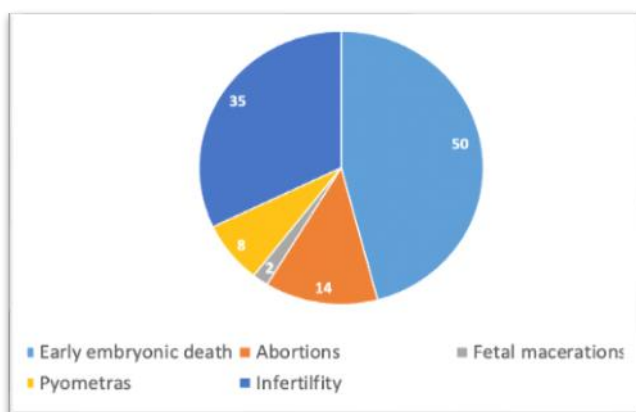


**Fig.1.** Structural features (a), Microscopic view (b) and Phylogenetic Status (c) of *T.foetus*

Trophozoites were typically found to reproduce through binary fission. Reference [10] has demonstrated a rare Pseudocyst form, upon exposure to any kind of stress such as nutrient deficiency, exposure to toxic drugs such as Griseofulvin, rapid thermal shifts etc.

### 3. Molecular Mechanism of Pathogenesis

In Cows, during natural infection, the trophozoites initially were found to colonize the skin folding in the cervical region. However, within 10-14 days they were found to disseminate the entire female genital tract, including the vagina, uterine endometrium, and oviducts [11]. *T.foetus*-induced inflammatory vaginitis with mucopurulent secretions was found to be the most prevalent symptom exhibited by the cows at the early stages of infection.



**Fig. 2.** Magnitude of different symptoms that occur in cows upon infestation with *T.foetus*

As estimated [12] the magnitude of *T.foetus* associated symptoms in cows are shown in **Fig. 2.** In the cases of natural service by an infected bull, it was reported that, over 30–90% of the total cows got infected in the herd. It was also found that, there were cases of parasite infection transmission from infected cows to bulls. [13, 14].

Based on the above understanding, screening of bulls is necessary before introducing them into the herd as well as after their mating in the herd. *T.foetus* was also found to get transmitted through contaminated semen during

Artificial Insemination. The transmission was documented in a few cases even during routine gynaecological examinations of cows during the use of contaminated equipment. Reference [13, 15] have demonstrated that *T.foetus* survives cryopreservation and hence were found to survive in the cryopreserved semen used for artificial insemination. Reference [16] had established that *T.foetus* infection results in the induction of several pathogenic effects such as stimulate the host cell lysis by secreting several Proteases, causing enhanced permeability at the tight junction, epithelial cell degradation and Apoptosis. *T.foetus* was found to possess several potential immunogenic proteins that could possibly involve in establishing the host-pathogen interaction and virulence [17].

### 4. Zoonoses

Several incidents of zoonotic transmission of *T.foetus* were reported in immunocompromised individuals exhibiting various serious symptoms like Pneumocystis pneumonia [18], Meningo-encephalitis [19,20], Peritonitis [21], and Cholecystitis [22] ,in immune-compromised individuals. *T.foetus* infection was found to be connected to a variety of characteristics such as age, breed, and herd management practices (“bull-to-cow ratio”).

The prevalence rates were higher in countries where traditional method of natural service was the predominant breeding method, while strict Implementation of Artificial Insemination (AI) had substantially decreased the burden in some regions of the USA [23]. Stringent implementation of regular screening programmes, maintenance of screening, , and restricting the entry of infected animals through proper maintenance of fencing for the herd were few control strategies implemented in Wyoming, USA, for complete eradication of *T.foetus* parasite. [24].

### 5. Geographical Distribution

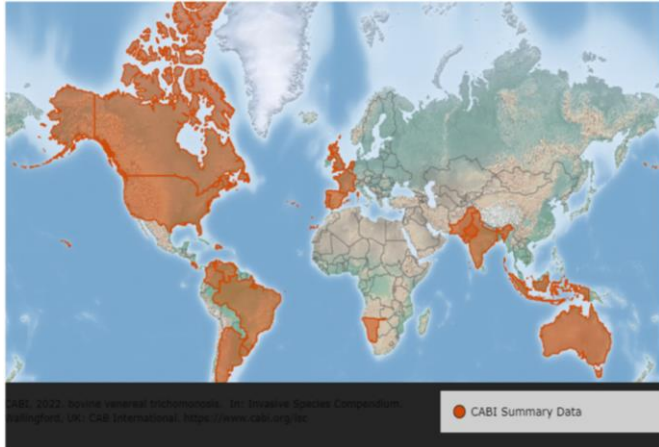
The first case of Bovine Trichomonosis (Also known as Trichomonosis) was documented in Pennsylvania as early as 1932 [25]. The disease exhibits worldwide prevalence of Bovine Trichomonosis (Fig.3).

*Trichomonas foetus* (*T.foetus*) (also referred as *Trichomonas fetus* /*Tritrichomonas foetus*), is the disease causing was found to be predominantly present in North America, South America, Southern parts of Asia, including INDIA. There were few sporadic cases reported from African and European countries.

Majority of the studies on Trichomonosis were mainly published from countries like United States, Canada, Argentina, Australia, France, Russia, Spain etc. There were very few studies conducted and reported related to screening Bovine Trichomonosis in INDIA.

A brief report on the prevalence of *T.foetus* infection across the world is summarized in Table.1. The Majority of these

estimates were based on findings related to single bull sample culture (rather than repeated sampling), small sample sizes and analysis of processes for collecting, preserving, and evaluating the cultured specimens in few reports raise the validity of the reported findings.



**Fig. 3.** Worldwide prevalence of Bovine Trichomonosis in cattle (Intensity of the colour indicates the relative level of prevalence) (CABI Web portal 2024)

**Table.1:** Data related to Worldwide Prevalence (%) of Bovine Trichomonosis in cattle estimated against modes of Breeding and Cattle type

| Country                                 | Cattle type      | Mode of breeding | Prevalence rate             | Reference |
|---|------------------|------------------|-----------------------------|-----------|
| Australia                               | Bulls            | Natural          | 65.9%                       | [26]      |
| Africa (north-western)<br>South Africa. | Bulls            | Natural          | 10-26.4%                    | [27]      |
| California<br>United states             | Beef             | Natural          | 4.1-15%                     | [28]      |
| Costa Rica                              | Cows and Bulls   | Natural          | 18.4%(Cows)<br>7.2% (Bulls) | [29]      |
| Rajasthan<br>India                      | Cows             | Natural          | 28.16%                      | [30]      |
| Argentina                               | Cows and heifers | Natural          | 3.5%                        | [31]      |
| Idaho<br>United states                  | Bulls            | Natural          | 40.9%                       | [32]      |
| Florida,<br>United states               | Bulls            | Natural          | 10% -<br>53%                | [33]      |
| Argentina                               | Herds            | Natural          | 28%                         | [34]      |
| Beijing,<br>China                       | Dairy herds      | Natural          | 5%                          | [35]      |
| Spain                                   | Bulls            | Natural          | 32%                         | [36]      |
| Texas<br>United states                  | Bulls            | Natural          | 3.7%                        | [37]      |
| Wyoming<br>United states                | Bulls            | Natural          | 2.69%                       | [38]      |
| Turkey                                  | Cows             | Natural          | 5.7%                        | [39]      |
| Argentina                               | Herds            | Natural          | 5.12%                       | [40]      |
| Bangladesh                              | Cows and Bulls   | Natural          | 1.5%                        | [41]      |
| U.P<br>India                            | Cows             | Natural          | 100%<br>(6 samples)         | [42]      |
| Brazil                                  | Cows and Bulls   | Natural          | 3.7%                        | [43]      |
| Argentina                               | Herds            | Natural          | 3.03%                       | [44]      |
| Wyoming,<br>United states               | Bulls            | AI               | 0%                          | [24]      |

## 6. Laboratory Diagnosis

As reported by [45] clinical symptoms associated with Trichomonosis were found to be difficult to distinguish from those of the most commonly reported sexually transmitted bacterial diseases such as Campylobacteriosis, Bovine TB, Leptospirosis, Brucellosis, as well as the most prevalent viral diseases transmitted through sexual transmissions such as IBR and BVD. Hence initially, several diagnostic methods like direct microscopy and culturing were developed for parasite detection directly in the clinical specimens. Polymerase chain reaction (PCR) and Real-time - Polymerase chain reaction (RT-PCR) based molecular diagnostics were developed for improved diagnosis. There were very limited attempts made towards developing serology-based onsite diagnostic assays.

### 6.1 Direct Microscopy

The experiments of [46] employed direct microscopy on a wet mount to show the presence of *T.foetus* parasites in clinical samples. Their findings revealed that organisms from clinical samples are better detectable when the samples were cultured in appropriate conditions to get an optimal number of  $10^4$ /mL. These are mostly detected in clinical samples by their pyriform appearance and jerky motions. Sometimes, no parasites were found in the samples that reach the laboratory at a delayed time.

Based on these findings, it was suggested that diagnosis is purely based on direct microscopy result in false negatives, and also there may be a high risk of confusion with morphologically similar commensal Trichomonads such as *Tetratrichomonas*, *Pentatrichomonas* etc. Reference [47] developed a rapid staining procedure known as Giemsa or Diff-Quick and iodine for detecting even a single parasite in a smear and [48] developed improved iodine staining and Giemsa staining procedures. Furthermore, the killing of parasites during fixation with the stain or loss of characteristic flashing movements would limit the detection of the parasites.

### 6.2. Culture

The composition of the medium, preparation method of the medium, way of sample collection and mode of transport were the most influencing factors for assessing the sensitivity of culture methods of *T.foetus*. For several years Diamond's trichomonad medium was the popular culture medium used for *T.foetus* detection [46]. InPouch TF-Bovine kits with the medium are commercially available and widely used for specific culturing of *T.foetus* (Biomed Diagnostics, USA). They were found to be suitable for sample collection, transport, culture, and direct microscopic evaluation [45].

Since the *T.foetus* are microaerophilic in nature, they tend to get accumulated at the bottom of the medium. Hence collecting the inoculum from the bottom of the transport medium serves as a good source for better sensitivity. *T.foetus* was observed to grow best at temperatures ranging from 30°C to 37°C. For microscopic detection of cultured

cells, the average incubation time was determined to be 2-3 days and in cultures, the trophozoites were generally found to survive for up to 7 days.

Light microscopy was extensively used for the identification of organisms, in a direct wet mount or by directly focusing the transparent pouch of the kit. It was recommended to view the sample mostly at the bottom of the culture medium as the parasites tend to accumulate at the bottom due to microaerophilic conditions. Trophozoites were mostly identified by their refractive pear-shaped body and their characteristic jerky movement at  $\times 40$  followed by  $\times 100$  (Biomeddiagnostics.com).

The sensitivity of Diamond's medium was estimated to vary from 78% to 99% [49] whereas the same for the Inpouch TF-Bovine culture kit was reported to be  $>92\%$  sensitive. As per the breeding regulations, three consecutive negative tests in three-week intervals were suggested to be suitable for declaring the animal as negative for *T.foetus*. In younger bulls of up to 6 months, a single negative test was considered sufficient [45].

### 6.3. Molecular Detection

DNA testing has become the gold standard method for the specific detection of *T.foetus*. Reference [23] reviewed that the sensitivity of PCR was superior to direct microscopy and culturing for the detection of *T.foetus* infection. PCR analysis was found to be influenced by the inhibitors of various sample matrices, however, it was found mandatory to optimize the reaction for each of the clinical samples.

Numerous molecular approaches to identify *T.foetus* DNA have been established mostly using conserved regions of the 5.8S ribosomal RNA gene and the flanking internal transcribed spacer regions (ITS) [50,51]. PCR and Real-time PCR-based molecular methods were found to offer numerous merits that include, higher sensitivity, specific identification of *T.foetus* in the presence of commensal trichomonads, shorter diagnostics turnaround time and non-requirement of viable cells etc.

Since the PCR reaction largely depends on the quality of template DNA, commercially available kits containing "magnetic beads and spin columns" were found to be of great use. However, the simple heat lysis method sample preparation was found to be sufficient for Real-time PCR reactions [52].

The first PCR reaction was developed by [53] using the primers viz, TFR3 and TFR4 to differentiate *T.foetus* from other commensal trichomonads of faecal contaminants. These primers were found to amplify 347bp DNA of *T.foetus*. Furthermore, an improved PCR was developed for detection process for differential diagnosis that uses one set (TFR1 and TFR2) for amplifying DNA from the trichomonad group and the *T.foetus*-specific set of primers TFR3 and TFR4 [50].

*T.foetus* DNA was shown to amplify DNA sequences using both the above sets of primers, whereas the

commensal Trichomonads could only amplify the sequences using TFR1 and TFR2 primers. Reference [54] employed TFR1 and TFR2 primers with TFR1 the forward primer in tag with a 6FAM fluorophore for refining the traditional PCR. Reference [52] developed an assay using the clinical samples that outperformed almost 10- folds of all previously developed PCR reactions employing the ITS-1 region.

The probe-based real-time PCR test identified even the presence of a single cell. This procedure significantly shortened the time required to prepare the sample for an assay. Initially though three positives were identified using culture/microscopic methods out of 159 clinical samples, only 14 samples were found to be positive indicating the sensitivity of the method.

A "loop-mediated isothermal amplification assay" (LAMP) was developed using recombinant DNA of 5.8S rDNA that was reported to have relatively superior sensitivity to the PCR method [55]. This assay was found to be simple to perform, as well as reported to be tolerant against the contaminants and inhibitors in the clinical samples over conventional PCR. However, LAMP is associated with cost due to the requirement of 10 primers and the complexity of designing primers. Similarly, reference [56] tested 833 samples collected from different parts of United States, first by culturing and then by real-time PCR. Another commercially available RT-PCR technique targeting beta-tubulin1 was developed.

Several refinements were done later to the previously developed PCR reaction. Reference [51] conducted a study using TFR3 and TFR4 primers along with DNA-chelating fluorescent dyes under real-time PCR conditions. Currently, there is only one commercially available kit, namely VetMAX™-Gold Trich Detection Kit, Thermo-Fischer Scientifics, USA) which is being widely used for Trichomonosis screening in several countries. A list of various primers used for PCR/RT-PCR is presented in Table. 2. Table.3 Summarizes the various molecular methods developed for *T.foetus* detection.



**Table.2.** Primers used against various target regions of *T.foetus* for different Molecular detection Assays for screening Bovine Trichomonosis in cattle

| Name of the Primer        | Sequence                         | Assay type       | Target region        | Ref. |
|---------------------------|----------------------------------|------------------|----------------------|------|
| TFR 3                     | CGGGTCTTCCTATA TGAGACAGAACC      | Conventional PCR | ITS1/5.8S rDNA/ ITS2 | [65] |
| TFR 4                     | CCTGCCGTTGGATCAGTTTCGTTAA        |                  |                      |      |
| TF211A                    | ACCT GCC GTT GGA TCA             | Conventional PCR | ITS1/5.8S rDNA/ ITS2 | [66] |
| TF211B                    | GTT TCG TTA, GCG CAA TCG         |                  |                      |      |
| TFR1                      | 5'GTAGGTGAACCTGCCGTIG3'          | Conventional PCR | ITS1 and ITS2 region | [50] |
| TFR2                      | 5'ATGCAACGTTCTTCATCGTG3'         |                  |                      |      |
| TFR1                      | 5'GCGGCTGGATTAGCTTTCTTT3'        | RT PCR           | ITS1/5.8S rDNA/ ITS2 | [52] |
| TFR2                      | 5'GGCGCGCAATGTGCAT3'             |                  |                      |      |
| TrichP2                   | 5'6FAMACAAGTTCGATCTTTGMGB3'      | LAMP PCR         | ITS1/5.8S rDNA/ ITS2 | [55] |
| TFR1                      | 5'GCGGCTGGATTAGCTTTCTTT3'        |                  |                      |      |
| TFR2                      | 5'GGCGCGCAATGTGCAT3'             |                  |                      |      |
| TFR 3                     | 5' CGGGTCTTCCTATA TGAGACAGAACC 3 |                  |                      |      |
| TFR 4                     | 5'CCTGCCGTTGGATCAGTTTCGTTAA3'    | RT PCR           | ITS1/5.8S rDNA/ ITS2 | [51] |
| Forward Internal Primers  | F1,F2,F3                         |                  |                      |      |
| Backward internal primers | B1,B2,B3                         |                  |                      |      |
| TFR 3                     | 5' CGGGTCTTCCTATA TGAGACAGAACC 3 | RT PCR           | ITS1/5.8S rDNA/ ITS2 | [51] |
| TFR 4                     | 5'CCTGCCGTTGGATCAGTTTCGTTAA3'    |                  |                      |      |
|                           | TaqMan-probe                     |                  |                      |      |

**Table. 3.** Data related to Molecular diagnostic methods for *T.foetus* detection

| Target                                     | Test                | Name of primer/probe                     | Reported specificity                                  | Reported sensitivity               | Ref. |
|--|---------------------|--|---|------------------------------------|------|
| ITS1/5.8S rDNA ITS2                        | PCR                 | TFR3, TFR4                               | <i>T.foetus</i> , <i>T.suis</i> & <i>T.mobilensis</i> | One or a few protozoa              | [2]  |
| TS1-5.8S rDNA-ITS2                         | PCR                 | Tricho-F/Tricho-R                        | Trichomonas & Pentatrichomonas                        | Used in human samples              | [18] |
| 5.8S rDNA                                  | RT- PCR             | (TFF2), (TFR2) & (5'FAM/3'MGB-NFQ) Probe | Trichomonas genus                                     | 3fg template, 1 cell/test          | [52] |
| ITS1/5.8S rDNA ITS2                        | PCR                 | TFR3, TFR4                               | <i>T.foetus</i> , <i>T.suis</i> & <i>T.mobilensis</i> | One or a few protozoa              | [53] |
| 5.8S rDNA                                  | LAMP                | LAMP primers                             | Specific to <i>T.foetus</i>                           | Ten cells/reaction                 | [55] |
| ITS1/5.8SrDNA ITS2                         | PCR                 | TFR1, TFR2                               | Trichomonas genus                                     | One or a Few protozoa              | [65] |
| 18S rDNA,ITS1,5.8S rDNA                    | PCR                 | TF211A,TF211B                            | <i>T.foetus</i> with few nonspecific DNA              | 1pg <i>T.foetus</i> DNA            | [66] |
| Probe designed based on conserved sequence | PCR & Southern blot | TF1, TF20.85 kb Synthetic probe          | Amplifies <i>T.foetus</i> <i>T. vaginalis</i>         | Ten or occasionally fewer protozoa | [67] |
| ITS1/5.8SrDNA ITS2                         | PCR                 | TFR1, TFR2                               | Trichomonas genus                                     | One or a Few protozoa              | [68] |

#### 6.4. Serological Techniques

Mucus agglutination test was developed by [57] for detection of *T.foetus* parasites from Cervico-vaginal mucus from naturally infected herds. 32% (57 of 178) were found to be non-reactive to co-existing bacteria such as *Campylobacter I* or *Brucella abortus*. Hemolytic assay [58] done with the serum of chronically infected bulls was found to show 94% sensitivity and 96% specificity. A bead agglutination assay using whole cell extracted antigen developed [59] was found to detect *T.foetus* cells present in the genital mucus. Further, few Indirect ELISA assays were developed using whole cell extract of the *T.foetus* cultured cells (Source of antigen) [60,61,62,63,64] against vaginal mucus, smegma as well as serum, for detection of *T.foetus* specific antibodies. However, they are yet to be validated for commercial application.

As per the evaluation of the developed diagnostic methods [45], Table.4 summarizes the Diagnostic methods

and their applicability for *T.foetus* detection. Based on the recommendations of OIE, Culture and a few PCR, RT-PCR-based diagnostic kits were developed for commercial use. However, their application was found to be quite limited due to the higher costs, requirement of technical expertise and longer duration for test results etc. Various diagnostic kits developed based on recommendations of OIE *T.foetus* are tabulated in Table. 5.

#### 7. Challenges

*T.foetus* identification by culture and microscopy is not specific, laborious, time-consuming, expensive, and needs professional competency. Preputial washings and sperm samples collected for testing have the potential to get contaminated easily hence posing to give false positive reports. On the other hand, all of the recently developed molecular detection technologies like PCR and RT-PCR indicate improved specificity, however the long turnaround time and the cost per individual test and the fact that they

require a complex lab setup and technical skills limit their scope of applicability in regular screening and surveillance programmes. Except for The VetMAX-Gold Trich Detection Kit, majority of kits (Table.5) were developed for research purposes only and are not authorized for commercial usage and need to be imported. Screening services are only accessible at a few regional and National institutions/research

laboratories, limiting their use in resource-limited field stations and not implemented at full potential[69].

Due to the cost associated with bulk culturing of *T.foetus*, long incubation periods and the zoonotic potential (human Transmission) associated with *T.foetus* cells, the earlier assays exhibited limited scope for commercialization.

**Table.4** List of Diagnostic methods and their applicability in Herd screening, Individual screening, Contribution to eradication Programmes, Evaluation of clinical cases, Agent prevalence – monitoring and Immunological status of cattle for *T.foetus* infection [42].

| Method            | Herd screening                                   | Individual screening                             | Contribution to eradication programs | Evaluation of clinical cases           | Agent prevalence monitoring                      | Immunological status |
|-------------------|--|--|--------------------------------------|--|--|----------------------|
| Direct Microscopy | Recommended & validated                          | Suitable<br>Need further validation              | Recommended and validated            | Recommended & validated                | Suitable<br>Need further validation              | NO                   |
| PCR               | Employed in certain settings<br>expensive        | Suitable<br>Need further validation<br>Expensive | Not applicable                       | Need further validation<br>Expensive   | Not applicable                                   | NO                   |
| PCR & culture     | Suitable<br>Need further validation<br>Expensive | Recommended & validated<br>Expensive             | Not applicable –                     | Recommended and validated<br>Expensive | Employed in certain settings<br>expensive        | NO                   |
| Real-time PCR     | Recommended & validated<br>Expensive             | Recommended & validated<br>Expensive             | Recommended & validated<br>Expensive | Recommended and validated<br>Expensive | Suitable<br>Need further validation<br>Expensive | NO                   |

**Table.5.** List of Various diagnostic kits developed for detection of *T.foetus* infection in cattle

| Kit description  | Intended use                    | Company                                | Technology          | Cost in Rs./test (Approx.) | Turnaround time |
|--|---------------------------------|--|---------------------|----------------------------|-----------------|
| ViPrimePLUS <i>Tritrichomonas I</i> Kit                        | Research                        | Vivantis Technologies, Malaysia        | qPCR                | 2000.00                    | 24h             |
| Inpouch TF-Bovine Culture                                      | Field Screening                 | Biomed diagnostics, USA                | Culture             | 500.00                     | 72h             |
| VetMAX-Gold Trich Detection Kit                                | Field Screening                 | Thermo fischer Scientific, USA         | Real-time PCR test  | 2000.00                    | 24h             |
| <i>Trichomonas I</i> gene Beta-tubulin 1 gene sig Advanced Kit | general laboratory and research | Genesig, UK                            | qPCR test           | 2000.00                    | 24h             |
| Techne qPCR test   | general laboratory and research | Thermo fischer Scientific, USA         | qPCR test           | NA                         | 24h             |
| <i>Tritrichomonas I</i> testing lab                            | Testing services                | University of Nebraska–Lincolnusa, USA | qPCR test & Culture | 500.00& 2000.00            | 72h 24h         |

### 8. Opportunities.

Trichomoniasis is very difficult to cure, the sole current approach is only screening and isolation of affected animals [70,71,72]. Despite the extremely low reproduction rate observed in cows, screening for sexually transmitted illnesses remains quite limited. The greatest impediment to regular screening tests is the lack of commercially available indigenous and simple diagnostic kits [73]. Hence, there is an immediate need for developing simple, cost-effective, easy-to-operate onsite usable Point-of-care diagnostic kits for individual farmers as well as organized farms. There is an urgent need to develop effective control strategies through vaccination [74].

As reported by earlier point-of-care testing (POCT) has been demonstrated to be more appropriate for on-site

rapid diagnosis, routine surveillance, monitoring, and management of disease at an earlier stage. The current value of the global POCT market is estimated to be approximately 43.2 billion USD, and by 2027, it is expected to reach 72 billion USD.

Nano technology based POC diagnostics for application in human medicine have made significant advances in recent times[75.76]. Among the several kinds of POCT, ELISA is used for bulk screening with a minimal laboratory set-up, whereas the lateral flow assay (LFA), a paper-based in-situ detection platform, is the most widely used rapid detection method in the field of biomedicine, environmental health, quality control, and food safety etc. [77].

## 9. Conclusions

While serological tests can screen animals quickly, molecular confirmation (PCR) can provide definitive diagnosis for borderline cases. A two-step approach (initial serological screening followed by confirmatory PCR) can increase overall diagnostic accuracy. Nanotechnology-based LFAs and newer detection chemistries can improve sensitivity and specificity. Smartphone-based readers for LFAs can capture quantitative data, store results, and integrate with herd-management software. Veterinarians, technicians, and farm workers should be trained on proper sample collection and interpretation of serological test results. Awareness of the disease's reproductive impact is key to ensuring producers adopt testing protocols. Widespread adoption of serological POCT will yield data on prevalence and geographic distribution of *T. foetus* in cattle populations. Continuous monitoring helps refine control measures and supports targeted vaccination or management strategies where available.

The recommendation to develop serological testing devices, such as iELISA and LFA rapid kits, is driven by the need for timely, efficient, and accessible diagnostic tools in the fight against Bovine Trichomonosis. Implementing these tests at the point of care can reduce the economic burden of reproductive losses, allow for better herd management, and ultimately support more comprehensive control strategies. Despite certain challenges—especially regarding assay validation, specificity, and field usability—these devices hold strong promise as essential components of modern cattle health management programs.

### Competing interests

Author declare no Competing interests.

### Data Availability

All the relevant data is provided in the manuscript.

### Conflict of Interest

Author declare that they do not have any conflict of interest.

### Funding Source

None

### Authors' Contributions

Dr. Geethanjali Karli researched literature and conceived the study, wrote and reviewed the entire manuscript.

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