ISJ 21: 82-98, 2024 ISSN 1824-307X

REVIEW

Detection and control of pébrine disease in the silkworm (*Bombyx mori*)

H Gu, Z Cao, Y Yang, K Chen, P Lü*

School of Life Sciences, Jiangsu University, Zhenjiang 212013, China

 Accepted August 30, 2024

Abstract

Pébrine disease in silkworms, caused by *Nosema bombycis*, significantly impacts silkworm reproduction and cocoon quality. Recognized as a legal quarantine concern in silkworm egg production, the damage from pébrine disease is on the rise. Currently, sericulture production relies on a combination of chemical and physical methods for pébrine control. Rapid and precise detection of *N. bombycis* is essential for effective disease management. Detection methods for microsporidia in silkworms include optical microscopy, electron microscopy, immunological methods, and molecular biology techniques such as PCR, qPCR, and LAMP technologies, along with biosensor-based methods. Despite their respective advantages, these methods face challenges in large-scale application. To effectively combat pébrine disease, future research must focus on developing innovative detection methods that are highly efficient, rapid, accurate, portable, and suitable for on-site instant detection of *N. bombycis*, thereby ensuring better control and swift detection of pébrine disease in silkworms.

Key Words: *Nosema bombycis*; pébrine disease; detection method; control method

Introduction

Microsporidia are specialized unicellular intracellular eucaryotic parasites (Long *et al.*, 2020) which were initially classified as Protozoa (Keeling *et al.*, 2000); however, systematic classification of microsporidia using molecular methods (Cheng *et al.*, 2021) has revealed that microsporidia are more closely related to fungi than protozoa (Keeling and Fast, 2002; Keeling, 2003; Lee *et al.*, 2008; Adl *et al.*, 2012). Microsporidia have a diameter of 1-40 μm with a three-layer structure of spore outer wall, spore inner wall, and protoplasm membrane. The cell wall is approximately 7 nm thick. The nucleus is usually circular or oval in shape, and the nucleus size varies with different life cycles. Compared with other eukaryotes, microsporidia have unique characteristics such as a lack of Golgi apparatus, peroxisomes, fully functional mitochondria and ribosomal RNA of the 70S type (Haag *et al.*, 2014). There are more than 1500 species of microsporidia belonging to 200 genera (Franzen, 2004; Franzen, 2005; Han *et al.*, 2021). Microsporidia parasitize host cells and infect a wide range of both invertebrate and vertebrate hosts including silkworms, shrimp, bees, fish and other economically important species. Insects are the most susceptible species to microsporidia infection. Invasion of the host by microsporidia usually causes lesions or adverse reactions in certain organs of the host, affecting the normal physiological activities and functions of the host. The pathogenicity and lethality of microsporidia to some economic species has caused significant losses. In summary, Microsporidia are ubiquitous pathogens that are important components of terrestrial and aquatic ecosystems worldwide and that have attracted attention from many scientific disciplines, including medicine, veterinary science, ecology, and evolutionary biology. This consideration led to the acquisition of genome data from many species of this phylum as a means to obtain an overview of their general biology. Phylogeny of Microsporidia species with complete genomes reconstructed using phylogenetic tree, based on the alignment of 19 orthologous protein-coding sequences, and characteristics of their genomes (Figure 1). With the continuous development of microsporidian detection technology, studies have shown that the human body is also one of the important parasitic hosts of microsporidia, which have brought about a serious impact on the life and health of human beings.

Pébrine disease in the silkworm (*Bombyx mori* L.) is an infectious disease caused by *Nosema bombycis* (Nb). This disease was first recognized in France in 1845, and it subsequently invaded the sericulture countries of Europe. Microsporidia infection in silkworms has a long incubation period and a wide range of transmission avenues including

Corresponding author:
Peng Lü
School of Life Sciences
Jiangsu University
Zhenjiang, Jiangsu Province 212013, PR China
E-mail: penglu@ujs.edu.cn

both horizontal and vertical transmission. It can be horizontally transmitted to silkworm larvae, pupae, and adults through peroral infection and vertically transmitted to offspring through the eggs (Li *et al.*, 2018; He *et al.*, 2019). The disease results in reduced fertility, decreased cocoon quality and great economic losses to production. *N. bombycis* is currently listed as the only legal quarantine object in sericulture, and it is also a key area of research in the sericulture industry (Fu *et al.*, 2016). To effectively prevent and control pébrine disease in silkworm production, a sensitive, accurate, rapid and convenient method for detection of *N. bombycis* is important. The successful detection of *N. bombycis* at the egg stage is also important for the prevention and control of the pébrine disease. In 1870, Pasteur established the classic adult female microscopy method, which is simple and rapid. However, its accuracy is affected by many factors, such as the working environment, the inspector's eyesight and the number of visual fields. Detection of *N. bombycis* in silkworms has now transitioned from traditional optical microscopy and electron microscopy to the immunological level, the molecular level and biosensor detection (Kajiwara and Murakami, 2019; Esvaran *et al.*, 2020; Jagadish *et al.*, 2021; Sivaprasad *et al.*, 2021; He *et al.*, 2022; Karunakar *et al.*, 2022; Kiani-Azad *et al.*, 2022; Moharrami *et al.*, 2022; Bagheri *et al.*, 2023). These technologies now play important roles in the prevention of pébrine disease in silkworms. This paper reviews research progress on the main detection techniques and control methods of *N. bombycis*. The information provides a reference and guide for the rapid and accurate detection and control of microsporidia in silkworms.

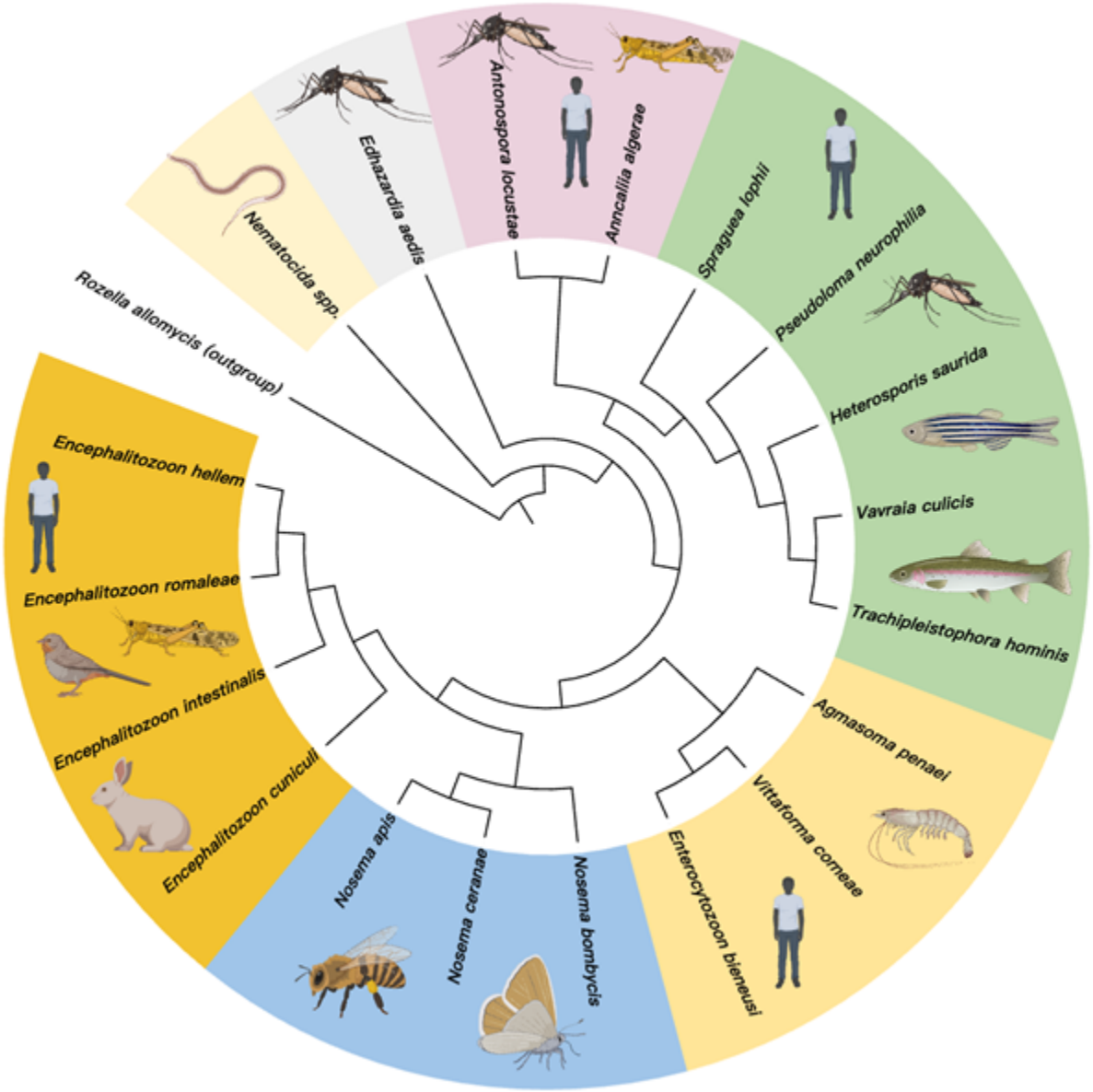

Fig. 1 Phylogeny of Microsporidia species with complete genomes

Table 1 Comparison of different dyeing methods

Year	Dyeing method	Target of dyeing	Color	Total time	References
1994	Chromotrope 2R	Spore	Red	90min	(Joseph *et al.*, 2006; Kokoskin *et al.*, 1994; Niu *et al.*, 2000)
1996	Chromotrop	Spore	Purple	10min	(Moura *et al.*, 1996)
1997	Acid-fast staining method	Spore	Pink	45min	(Ignatius *et al.*, 1997)
1997	Uvitex 2B	Spore	Blue-white	5-10min	(Ignatius *et al.*, 1997)
1997	MTS-Uvitex 2B	Spore	Blue-white	5-10min	(Ignatius *et al.*, 1997)
2002	$KMnO_4$-methyl violet	Spore	Purple	5-10min	(Prasa *et al.*, 2000; Tang *et al.*, 2002)
2006	KOH+CFW	Spore	Bluish blue	5-10min	(Joseph *et al.*, 2006)
2007	Calcofluor M2R	Spore	Bluish blue	5-10min	(Liu and Zeng, 2007)
2011	Gram-chromotrope kinyoun (GCK)	Spore	Red	5-10min	(Salleh *et al.*, 2011)
2017	Fluorescent brightener	Spore	Blue	5-10min	(Dai *et al.*, 2017)
2017	Propidium iodide	nucleus	Red	5-10min	(Dai *et al.*, 2017)

Research progress of detection methods for *N. bombycis*

Sampling inspection of female moths is the most important part of the detection of *N. bombycis* in silkworms, but its accuracy is affected by many factors, which may lead to unqualified silkworm eggs entering the market and causing unnecessary economic losses (Yan *et al.*, 2014; Dai *et al.*, 2017). Researchers have conducted extensive exploration and research on the detection methods of microsporidia, developing from the earliest naked eye identification to optical microscopy, electron microscopy, immunological testing, and molecular biology-based detection methods (Stine *et al.*, 2005; Liu *et al.*, 2008; Lee *et al.*, 2010; Sanders and Kent, 2011; Maikai *et al.*, 2012), which effectively guarantee the quality of silkworm eggs and improve the economic benefits of sericulture.

Traditional detection methods

Microscopy methods

N. bombycis can parasitize various silkworm stages including the larvae, pupae, adults and eggs. The naked eye identification method involves recognizing larvae with dark (or pale) rust colored bodies, sluggish movement, decreased appetite, and stunted growth. This method is widely used in sericulture production due to its simplicity, practicality and low equipment requirements. However, its accuracy depends on the skill of the test personnel and it cannot accurately detect pébrine disease at the early stage of silkworm growth. Therefore, it can only be used as a judgment basis for the middle and late stages of silkworm infection. The detection of *N. bombycis*, based on microscopic examination technology, is often used. This approach predominantly observes spores by optical microscopy and polar filaments and other species-specific ultrastructural features of microsporidia by transmission electron microscopy (TEM). The most commonly used method for microsporidia detection in silkworm eggs is the microscopic detection of adult females technique established by Pasteur (Cao *et al.*, 2016). This uses optical microscopy to determine if adult females carry microsporidia and, if so, blocks transovarial transmission of pébrine disease. However, this method has a low detection rate and a long detection period. Also, the primary color microscopy of female moths is susceptible to interference from other species such as *Nomuraea rileyi* and *Aspergillus flavus*. To address these problems microscopy has been combined with staining methods. Commonly used stains now include Giemsa stain, Gram's stain, and propidium iodide stain (Table 1) (Dai *et al.*, 2017). Combining microscopy with staining makes the detection process faster and simpler. However, its sensitivity is still low, making it prone to error, and the staining method cannot distinguish the specific species of microsporidia (Corcoran *et al.*, 1995). Accordingly, the recognition and identification of microsporidia species needs to use electron microscopy as well as modern immunological and molecular biological techniques (Corcoran *et al.*, 1995; Moharrami *et al.*, 2022).

Table 2 Immunological method assays for the detection of *N. bombycis*

Methods	Detection targets	Sensitivity	Detection time	Advantages	Disadvantages	References
IGSS	IgG	1.0×10^5 spores/mL	30min	● Reduce the interference of endogenous enzymes of silkworm and moth	● Too many operation steps ● Low specificity and practicability ● The color rendering effect is not obvious	(Liu *et al.*, 1995)
IFA	2G10 2B10	1.0×10^8 spores/mL	1-2h	● High specificity	● Cumbersome operation ● Time-consuming ● Low sensitivity	(Chen *et al.*, 2017; Huang *et al.*, 2018; Li *et al.*, 2007; Li *et al.*, 2006)
Western blotting	2G10 2B10	1.0×10^6 spores/mL	4-5h	● High sensitivity and specificity	● Time-consuming ● High technical requirements ● Expensive and prone to false-positives	(Chen *et al.*, 2017; Huang *et al.*, 2018; Li *et al.*, 2007)
ELISA	IgG	5.0×10^6 spores/mL	3-4h	● High sensitivity and specificity ● Ease of use and simple ● Wide range of applications ● Capable of qualitative and quantitative analysis	● Poor repeatability ● Prone to false-positives ● Time-consuming	(Chen *et al.*, 2017; Huang *et al.*, 2018; Li *et al.*, 2007; Li *et al.*, 2006; Wan M, 2008)
CGIS	SWP32	8.0×10^6 spores/mL	15min	● Ease of use and rapid ● Naked eye visual observation ● Low cost and high specificity	● Low sensitivity ● Not high throughput	(Zhou, 2014)

Immunological method

The immunological detection of microsporidia is characterized by strong specificity and high sensitivity, involving specific binding reactions of antigen and antibody, as well as immune amplification technology. Table 2 shows various immunological detection methods that have been developed, including immunoassay (IA), indirect immunofluorescence (IFL), enzyme-linked immunosorbent assay (ELISA), counter immunoelectrophoresis (CIEP), and western blotting analysis. These methods mainly target IgG and IgM antibodies (Garcia, 2002), as well as polyclonal and monoclonal antibodies against *N. bombycis* prepared using the spore surface antigen, spore wall proteins and some recombinant proteins such as HSWP5, NbSLP1, NbSLP1P, and NbSLP1C (Sivaprasad *et al.*, 1997; Cai *et al.*, 2011; Dang *et al.*, 2013).

Monoclonal antibodies and polyclonal antibodies against spores such as *N. bombycis* and *Encephalitozoon spp*. have now been successfully developed (Mo and Drancourt, 2004) and used to test microsporidia in clinical samples (Visvesvara *et al.*, 1994). Liu et al. applied a monoclonal antibody against the silkworm pébrine disease, together with immunogold-silver staining (IGSS), to detect *N. bombycis* at the light microscope level, which can distinguish spores from other tissues (Liu *et al.*, 1995). By combining monoclonal antibodies with immunochromatographic technique, Zhou et al. prepared a colloidal gold immunoassay strip (CGIS) for identifying *N. bombycis*, with a detection sensitivity of 8.0×10^6 spores/mL (Zhou *et al.*, 2014). Li et al. developed an immunofluorescence assay (IFA) for the detection of *N. bombycis*, which is fast, specific, sensitive, stable, and easy to conduct (Li *et al.*, 2006). Li et al. confirmed that ELISA, IFAT, and immunogold silver staining of monoclonal antibodies based on western blotting can specifically distinguish *N. bombycis* proteins (Li *et al.*, 2007). IFA, ELISA, and western blotting require the use of antibodies to identify characteristic pathogens in silkworm tissues and *in situ* infections on fixed specimens. However, these methods use fluorescence microscopy examination, and some antibodies exhibit cross reaction to different types of microsporidia (Chen *et al.*, 2017; Huang *et al.*, 2018). Wan et al. developed a dual antibody sandwich ELISA method for detecting *N. bombycis*, with a minimum detection amount of 3.1×10^5 spores/mL for purified spores and 5.0×10^6 spores/mL for infected silkworm eggs (Wan *et al.*, 2008). These immunological methods can also be used for the identification of other types of microsporidia and have good detection performance (Mo and Drancourt, 2004; Sheoran *et al.*, 2005; Omura *et al.*, 2007; Izquierdo *et al.*, 2017; Nagai *et al.*, 2019; Chozas *et al.*, 2023).

Monoclonal and polyclonal antibodies have good efficacy in diagnosing and distinguishing clinical samples of different types of microsporidia, but they have drawbacks such as cross-reactions, high proportions of false-positive reactions, expensive fluorescence microscopy, and lower sensitivity in identifying microsporidia than light microscopy staining (Schwartz *et al.*, 1994). Hence, difficulties and challenges remain in using

immunological techniques to distinguish and diagnose microsporidia. To address these issues, antibodies should be stained and labelled to improve the sensitivity and specificity of the immunological diagnosis.

Modern molecular biological detection methods

With the significant progress of modern molecular biology technology, detection methods have evolved from morphological observation and physiological and biochemical characteristic detection to molecular biology detection with higher sensitivity and greater specificity. The commonly used molecular biology detection methods mainly comprise nucleic acid hybridization technology, gene chip technology, PCR (polymerase chain reaction), quantitative real-time PCR (qPCR), loop mediated isothermal amplification (LAMP) and other molecular biology technologies based on the amplification of nucleic acid sequences of target genes. These methods are convenient, rapid and highly automated. They can meet the needs of modern clinical pathogen detection and have great application prospects. With the successful sequencing of more than 100 species of microsporidia, the use of molecular biology techniques to detect microsporidia has become routine. PCR, qPCR, and LAMP methods are the most common methods for detecting microsporidia (Verweij *et al.*, 2007; Bellstedt *et al.*, 2010; Ravikumar *et al.*, 2011; Yan *et al.*, 2014; Liu *et al.*, 2015; Choi *et al.*, 2016; Fu *et al.*, 2016; Wu *et al.*, 2017; Zhao *et al.*, 2017; Xie *et al.*, 2018; Dai *et al.*, 2019; Esvaran *et al.*, 2019; Sivaprasad *et al.*, 2021). These detection methods predominantly make use of specific primers to amplify *SSU rRNA* (small subunit ribosomal RNA), *LSU rRNA* (large subunit ribosomal RNA), *IGS* (intragenic spacers), *PTP* (polar tube protein, e.g. PTP1, PTP2, PTP3), *SWP* (spore wall protein, e.g. SWP5, SWP8) and related genes. Multiple molecular detection methods have been developed for the *SSU rRNA* gene and the variable regions of *SSU rRNA* gene can also be used to investigate the evolutionary relationships among microsporidia species. It should be pointed out that *SSU rRNA* gene exists in multiple copies in the genome, which makes its quantification of microsporidia less ideal (Klee *et al.*, 2006; Liu *et al.*, 2013; Bateman *et al.*, 2016).

PCR detection methods

PCR technology is commonly used because of its simplicity, small amount of sample required, high specificity and high sensitivity. Chen et al. designed two pairs of specific primers based on the *SSU rRNA* gene sequence of *N. bombycis* and *Vairimorpha*, and performed PCR detection for *N. bombycis* and other infectious microsporidia (Chen *et al.*, 1996). Their results indicated that primer I is a highly specific primer for detecting *N. bombycis*, while primer II can also be used for detecting microsporidia. Liu et al. designed universal primers for detecting pébrine disease by comparing the *SSU rRNA* sequences of six microsporidia, including *N. bombycis*, *Nosema antheraea*, *Nosema sp.*, *Nosema hemerophila*, *Nosema sp.* PA and *Nosema sp.* MPr (Liu *et al.*, 2009). The results confirmed that the designed PCR primers specifically amplified the six types of microsporidia mentioned above. Through further improvement and optimization, PCR detection of microsporidia in different life stages (eggs, larvae, pupae, adults) of silkworms can be achieved (Liu *et al.*, 2009), which will have a positive effect on the quarantine and prevention of pébrine disease in the future. Bellstedt et al. developed a direct PCR (dPCR) method to amplify DNA directly from tissue samples of *N. bombycis* (Bellstedt *et al.*, 2010). This eliminated the need for DNA isolation and purification, and saved experimental time and cost. Multiplex PCR (mPCR), with the characteristics of being fast, economical, and high-throughput, can be used for the early detection and simultaneous detection of several microsporidian pathogens causing pébrine disease (Hatakeyama and Hayasaka, 2003). This has future practical value for the diagnosis and analysis of pébrine disease. Furthermore, the newly developed PCR-NALFS (nucleic acid lateral flow strips) has good specificity for common microsporidia isolates in silkworms, and the detection results can be directly read by eye. Importantly, this method can directly detect silkworm eggs and minimize false positives through indirect detection (Huang *et al.*, 2004; He *et al.*, 2019;). However, further improvements in the sensitivity of the NALFS method are possible.

As previously described, traditional PCR methods and their extended techniques can detect the DNA of microsporidia at all stages of their life cycle and differentiate parasites using specific primers (Refardt and Ebert, 2006). Compared with visual recognition alone under a microscope, their detection sensitivity and specificity have been greatly improved. However, due to the dependence of PCR amplification on specialized thermal cyclers, it is unsuitable for detection under field conditions. In addition, if the designed primers have weak specificity, false-positive results can occur.

qPCR detection methods

Although PCR methods have been widely used for the detection of *N. bombycis* (Hatakeyama and Hayasaka, 2003; Liu *et al.*, 2015), PCR methods cannot quantify the level of spores in a single reaction. Quantitative real-time PCR (qPCR) can achieve amplification and detection in one step, which can be both qualitative and quantitative. Therefore, it can be used for the detection and quantification of a wide range of pathogens, including human pathogenic microsporidia (Wolk *et al.*, 2002; Menotti *et al.*, 2003; Verweij *et al.*, 2007; Polley *et al.*, 2011) and agricultural microsporidia (Bourgeois *et al.*, 2010; Erler *et al.*, 2012; Chaimanee *et al.*, 2013). This has considerable advantages in sericulture quarantine work (Table 3). Fu et al. designed specific primers according to the conserved region of *SSU rRNA* gene in *N. bombycis*, and used qPCR to detect and quantify microsporidia in single silkworm eggs and newly-hatched larvae (Fu *et al.*, 2016). This method had high accuracy, high potential for high throughput screening, and was expected to be applied for the detection of microsporidia in sericulture production (Fu *et al.*, 2016). Wu *et al*. developed a multiplex real-time

Table 3 PCR and qPCR assays for the detection of *N. bombycis*

Methods	Detection targets	Sensitivity	Detection time	Advantages	Disadvantages	References
[a] PCR	SSU rRNA	$3\times10^{2-3}$ spores/mL	4-5h	● High specificity ● Simple and convenient	● Time consuming ● Poor quantitative ability	(Chen *et al.*, 1996; He *et al.*, 2011; Liu J *et al.*, 2009; Liu *et al.*, 2004)
[a] Direct PCR	SSU rRNA	$2\times10^{2-3}$ spores/mL	2-3h	● Simple, fast, and easy to perform ● Suitable for large sample numbers ● Cost-effective	● Poor quantitative ability	(Bellstedt *et al.*, 2010)
[a] Multiplex PCR	SSU rRNA	$1\times10^{2-3}$ spores/mL	2-3h	● The potential for high throughput screening ● Cost-effective	● Low stability ● Poor quantitative ability	(Hatakeyama and Hayasaka, 2003)
[b] Multiplex qPCR	SSU rRNA	8.5×10^{3} copies/μL	4-5h	● The potential for high throughput screening ● High sensitivity and specificity	● Time consuming ● Poor quantitative ability	(Wu *et al.*, 2017)
[b] SYBR-green qPCR	β-tubulin	100 pg/μL	2-3h	● Convenient and rapid ● Less prone to contamination and high sensitivity ● No need for electrophoresis	● Unable to distinguish different species ● Relatively low specificity	(Esvaran *et al.*, 2019; Fu *et al.*, 2016; Ironside, 2013; Taniuchi *et al.*, 2011)
[b] TaqMan-qPCR	β-tubulin	6.9×10^{2} copies/μL	2h	● High sensitivity and specificity ● The ability to distinguish different species ● Good quantitative ability and practicality ● Suitable for field testing	● Expensive ● Only suitable for a specific targ gene	(Alía *et al.*, 2020; Jagadish *et al.*, 2021; Nagy *et al.*, 2017; Tajadini *et al.*, 2014; Wang *et al.*, 2020)
[c] PCR-NALFS	LSU rDNA	1 pg/μL	2-3h	● Ease of use, cheap, and rapid ● High sensitivity and specificity ● Naked eye visual observation	● Poor quantitative ability ● Require tedious DNA extraction	(He *et al.*, 2019)

Note: a: Require PCR and electrophoresis instruments; b: Require quantitative PCR instruments; c: Combine PCR technology and NALFS technology

PCR (multiplex-qPCR) method (Wu *et al.*, 2017). Compared with traditional PCR, the multiplex-qPCR method has strong specificity, high sensitivity and high throughput. It can simultaneously detect three pathogenic bacteria (*N. bombycis*, BmNPV, and BmDNV) in silkworms (Wu *et al.*, 2017). The *rRNA* gene is a detection target for various microsporidia and microsporidia strains are usually classified according to their rRNA sequences (Ironside, 2013). The highly conserved eukaryotic *β-tubulin* gene is an early gene expressed together with the reported 16*s rRNA* gene, which has been successfully used for detecting microsporidia in wild and commercial silkworms (Taniuchi *et al.*, 2011; Esvaran *et al.*, 2019). Esvaran et al. developed a wide range qPCR method based on SYBR-green fluorescent dye for the detection of the *β-tubulin* gene of *N. bombycis* (Esvaran *et al.*, 2019). They found that the expression level of *β-tubulin* gene varied in different infection stages and tissues of silkworms, with the highest expression level in the midgut, followed by the ovaries, malpighian tubules, eggs, and fat bodies. These data indirectly indicated that the main infection pathway of microsporidia is oral transmission (Fu *et al.*, 2016). The high expression level in the ovaries confirmed that the infection of *N. bombycis* could be transmitted through the ovary, and the expression of *β-tubulin* gene in eggs revealed that the infection could spread from the midgut to the eggs, as well as from the adult females to their offspring.

The above methods have strong specificity and provide quantitative detection, but they cannot distinguish different types of microsporidia. Jagadish et al. used TaqMan probe qPCR to identify specific microsporidia (Jagadish *et al.*, 2021). They also found that the infection level of *N. bombycis* in the silkworm midgut was higher, followed by ovaries, malpighian tubules, eggs, and fat bodies, further confirming vertical transmission (Jagadish *et al.*, 2021). The TaqMan qPCR method has been widely used for the detection of viral and bacterial pathogens in humans, animals and plants (Nagy *et al.*, 2017) as well as for genotyping, mutation quantification and gene expression profiling (Tajadini *et al.*, 2014; Alía *et al.*, 2020; Wang *et al.*, 2020). Although qPCR can quantify infected silkworm pathogens and has advantages such as

high specificity, sensitivity, and low false-positives, it requires expensive equipment and it is difficult to widely promote and apply in real-world production practice.

LAMP detection methods

Loop mediated isothermal amplification (LAMP) is an in vitro method of DNA isothermal amplification that overcomes the PCR and qPCR need for sophisticated thermal cyclers. This greatly improves the speed and sensitivity of detection and makes molecular diagnosis simpler and more suitable for on-site detection (Notomi *et al.*, 2000). LAMP is now widely used for detection of many pathogenic microorganisms (Njiru *et al.*, 2008; Techathuvanan *et al.*, 2010; Jiang *et al.*, 2012; Singh *et al.*, 2013;). Liu et al. designed LAMP primers for the *EB1* gene related to spore reproduction and replication to detect *N. bombycis* (Mori and Notomi, 2009; Liu *et al.*, 2015). This could save nearly 1 hour compared to the conventional PCR method, with 100-fold higher sensitivity and without the need for expensive instruments. LAMP technology has also been continuously improved, and a variety of LAMP variants have emerged for the detection of *N. bombycis* and other microsporidia (Table 4) (Mori and Notomi, 2009). Sivaprasad et al. developed and validated a method named field friendly loop-mediated isothermal amplification (FF-LAMP), which eliminated the extraction and purification of target gDNA and did not require precision instruments (Sivaprasad *et al.*, 2021). The products can be detected using a colorimetric system, making it suitable for rapid on-site detection of large numbers of samples in sericulture production. Xie *et al*. constructed a LAMP-pH meter platform, which offered the advantages of high specificity, sensitivity, cost-effectiveness and quantifiability (Xie *et al.*, 2014). This is significant for the detection of *N. bombycis* and the development of portable detection equipment. Dai et al. integrated LAMP technology with gold nanoparticles (AuNPs) to display amplified products using AuNP colorimetry (Dai *et al.*, 2019). The LAMP-AuNP method has simple operation steps and low cost, making it highly suitable for the rapid detection of *N. bombycis* in small laboratories and production fields. LAMP-AuNP technology has been successfully applied to on-site detection of *shrimp yellow head virus* and *Enterocytozoon hepatopenaei* (Jaroenram *et al.*, 2012; Suebsing *et al.*, 2013). However, the applicability of the LAMP-AuNP method for on-site detection of *N. bombycis* requires optimization of detection conditions. Table 4 provides a detailed list of commonly used LAMP assays and their variants.

Biosensor detection methods

As previously mentioned, molecular biology methods, including PCR, qPCR, and LAMP, have been used to accurately identify *N. bombycis*. However, the detection and diagnosis of pébrine disease requires further improvement in sensitivity and specificity. While being suitable for on-site detection, it also needs to be more rapid and convenient, cost-effective and more efficient. These requirements have led to the development of innovative biosensor detection methods that combine multiple technologies (Table 5).

Electrochemical proximity analysis (ECPA) is similar to both electrochemical DNA sensing and aptamer-based protein sensing. It uses a proximity effect to guide electrochemical active markers towards, or away from, the electrode in the case of two probes binding to protein targets (Ferapontova *et al.*, 2008; Tong *et al.*, 2011; Yu and Lai, 2013). Signal amplification is the key to improving the performance and sensitivity of protein electrochemical detection. The most commonly used method is to use enzymes for electrocatalytic amplification. For example, horseradish peroxidase (HRP) (Mani *et al.*, 2009) was used to detect cancer biomarker proteins, and glucose oxidase (GOD) (Han *et al.*, 2012) was used to amplify electrochemical signals in thrombin detection. However, one disadvantage is that proteases are expensive and labile. Therefore, DNA enzymes are now being used for signal amplification because of their chemical stability, cost-effectiveness, and ease of synthesis and modification compared to proteases (Breaker, 2000; Willner *et al.*, 2008). Based on the characteristics of electrocatalytic reduction of Fe_3O_4 nanoparticles (Fe_3O_4NPs) to small molecule dyes (Zheng *et al.*, 2014), Wang et al. used the ECPA system as the basis, Fe_3O_4NPs as the catalyst, and the electrocatalytic reduction electron medium methylene blue (MB) for signal amplification to detect the total protein of *N. bombycis* (TP N.b) (Wang *et al.*, 2016). This method has the advantages of flexibility and simplicity in proximity analysis, as well as high sensitivity in electrochemical detection. It also overcomes the drawbacks of proteases in electrocatalytic signal amplification, shortens the interaction distance between catalysts and detection substances and greatly improves electrocatalytic efficiency. Therefore, the ECPA system with the function of signal amplification provides the possibility for quantitative analysis of many other types of protein biomarkers, and also has broad application prospects in real-time detection and microanalysis of proteins. Artificial mimic enzymes with catalytic abilities similar to peroxidase can also serve as catalysts for signal amplification in electrochemical biosensors, which can effectively catalyze the oxidation of methylene blue, thionine, and ferrocene mediated by H_2O_2, and enhance electrochemical signals (Zhou *et al.*, 2013). Willner et al. found novel catalytic activity of hemin/G-quadruplex as an DNAzyme, which can catalyze the oxidation of thiols to their respective disulfides during the in-situ generation of H_2O_2 (Willner *et al.*, 2008). Based on these findings, Wang et al. used the spore wall protein of *N. bombycis* as the target protein, L-cysteine as the stable electrocatalytic substance, and C_{60}@Pt-Pd nanoparticles as the support carrier to construct a hemin/G-quadruplex induced pseudo-double enzyme electrochemical biosensor (Wang *et al.*, 2014; Wang *et al.*, 2015). This electrochemical biosensor possesses high sensitivity and provides a rapid and sensitive diagnostic method for detecting *N. bombycis* (Wang *et al.*, 2014; Wang *et al.*, 2015).

Table 4 LAMP assays and its variants for the detection of *N. bombycis*

Methods	Detection targets	Limit of Detection	Detection time	Advantages	Disadvantages	References
LAMP	EB1	1.0×10^2 copies/μL	90min	● Low cost ● Ease of use and rapid ● Limited pre-sample processing ● Naked eye visual observation	● Complicated primer design ● Low efficiency of DNA extraction ● Weak quantitative ability ● Non-discrimination between pathogenic and non-pathogenic microsporidia	(Liu *et al.*, 2015; Mori and Notomi, 2009)
FTA-LAMP	LSU rRNA	10 spores/mL	45-60min	● Ease of use and rapid ● High efficiency of DNA extraction	● Complicated primer design ● Unable to distinguish different species ● Weak quantitative ability	(Huang *et al.*, 2004; Keeler *et al.*, 2012; Pan *et al.*, 2007; Subrungruang *et al.*, 2004; Yan *et al.*, 2014)
pH-LAMP	PTP1	0.5 fg/μL	45-60min	● Low cost ● Ease of use and rapid ● Good quantitative ability ● Portable testing equipment	● Require certain operational skills ● Require electrochemical equipment ● The problem of signal resolution	(Salm *et al.*, 2014; Toumazou *et al.*, 2013; Zhang *et al.*, 2014)
ATP-LAMP	PTP1	0.47 fg/μL	45-60min	● Low cost ● Ease of use and rapid ● Portable testing equipment	● Require certain operational skills ● Require electrochemical equipment ● Complex design of aptamers ● Need more materials	(Guo *et al.*, 2015; Jansson and Jansson, 2002; Sun *et al.*, 2010; Xie *et al.*, 2018; Xie *et al.*, 2015)
AUNP-LAMP	SSU rRNA	10 spores/mL	65min	● No cross reaction ● Ease of use and rapid ● Simple and portable equipment ● Suitable for small laboratory, field testing	● Complex synthesis and modification of nanoparticles	(Dai *et al.*, 2019; Jaroenram *et al.*, 2012; Suebsing *et al.*, 2013; Wang and Ma, 2009)
FF-LAMP	SSU rRNA	10 spores/mL	45-60min	● Without DNA extraction and purification step ● More suitable for field testing	● Weak quantitative ability	(Sivaprasad *et al.*, 2021; Toumazou *et al.*, 2013; Xie *et al.*, 2014)

The above methods provide new approaches for the detection of pébrine disease, as well as new ideas for the detection of other pathogens. However, due to the lack of direct, interpretable results, they are rarely applied for on-site testing. Other advanced modern analytical tools are now being used to identify *N. bombycis*. For example, Kajiwara et al. used MALDI-TOF MS (matrix-assisted laser desorption/ionization time of flight mass spectrometry) to quickly distinguish and identify different microsporidia in silkworms (Kajiwara and Murakami, 2019). The application of mass spectrometry has become a popular method for microbial identification (Hatakeyama and Hayasaka,

2003; Sauer *et al.*, 2008). In the future, MALDI bio-typing may become the first choice for insect disease diagnosis and a powerful analytical and testing tool in life sciences (Buchan and Ledeboer, 2014; Kajiwara *et al.*, 2016; Kajiwara and Murakami, 2019; Spanu *et al.*, 2012; Zhou *et al.*, 2017). The advantages and disadvantages of the newly developed biosensor and MALDI-TOF MS technologies are listed in Table 5.

Research progress of control methods for pébrine disease

The prevention and control of *N. bombycis* is a key and difficult area in the sericulture industry. At present, according to the characteristics of horizontal and vertical transmission of microsporidia, the prevention and control methods developed in sericulture production are to detect the presence of microsporidia in adult females through microscopy to interrupt vertical transmission, and prevent horizontal infection of microsporidia during the sericulture period. At the same time, under the prerequisite of placing prevention first and combining with control, chemical and physical methods are also primarily used to prevent and control the pébrine disease.

Conventional control methods

According to the occurrence and development laws of pébrine disease, the key to prevention and control is to eliminate transovarial transmission, strictly prevent foodborne transmission, and produce non-toxic silkworm eggs. In the sericulture industry, the production supervision regulations should be strictly implemented, and the microscopic inspection of adult females, pre-examination and remedial inspection should be carried out. The silkworm breeding department should strictly implement the national regulations on the microscopic examination of adult females, and conscientiously carry out the microscopic examination of bagged moths and stored moths. For female moths carrying microsporidia, their eggs should be eliminated according to regulations. The management of imported silkworm eggs ought to be strengthened and make sure that there is no pébrine disease before large-scale rearing of silkworms. Meanwhile, it is necessary to strictly select the silkworm rearing area, investigate the distribution of pathogens in the environment, mulberry garden conditions, sericulture equipment, and strengthen the management and technical guidance of the silkworm rearing area. Furthermore, during the sericulture period, disinfection methods are used to prevent foodborne transmission. The disinfection of silkworm rooms and tools before sericulture should be strictly carried out, and the silkworm rearing bed and silkworm body should also be regularly sterilized to cut off the source of infection and ensure the safety of sericulture. The weak and sick silkworms should be strictly abandoned, and diseased silkworms should be concentrated disinfected and deeply buried. The silkworm excrement should be promptly removed to prevent the spread and contamination of pathogens. Additionally, prevention and control of pests in mulberry field and disinfection of mulberry leaves should be reinforced, and cross infection of wild poisonous insects with silkworms must be eradicated.

Physical control methods

Physical prevention and control of pébrine disease mainly includes ultraviolet disinfection, high-temperature immersion, and high-temperature steam (Huang *et al.*, 2011; Huang *et al.*, 2016; Xing *et al.*, 2018; Li *et al.*, 2023). Soaking silkworm eggs in hydrochloric acid at 45-47 °C for 6-10 minutes will sterilize the egg pathogens. This reduces the pathogen incidence rate and helps prevent and control pébrine disease. Xing *et al*. demonstrated that 20 h-egg stage silkworm eggs treated with hydrochloric acid at a concentration of 1.075 g/mL and a high temperature of 48 °C for 6 minutes effectively prevented the occurrence of pébrine disease (Xing *et al.*, 2018). Because the host has greater tolerance to high temperature than *N. bombycis*, it is possible to kill or inhibit the schizonts or spores of *N. bombycis* at a certain temperature without harming the silkworm eggs. Based on the temperature tolerance, together with a high-temperature steam method, Wang et al. found that treatment with steam at 60 °C for 30 min can effectively reduce the incidence rate of pébrine disease. The treatment effect of pébrine disease transmitted transovarially is 97.25%, and has no adverse effects on egg hatchability, larval feeding and cocoon quality (Xing *et al.*, 2018). The results of fixed-point production experiments in rural areas showed that silkworm eggs treated with high-temperature steam can significantly reduce the incidence of pébrine disease in newly-hatched silkworms and 1-3rd instar late silkworms, and improve the yield and quality of silkworm cocoons (Xing *et al.*, 2018). Huang et al. confirmed that irradiation with a 20 w ultraviolet lamp at a distance of 50 cm for more than 12 minutes can effectively kill the microsporidia in silkworms (Huang *et al.*, 2011). However, ultraviolet radiation impacts the genetic material and immune system of silkworms and other organisms, and it can only be used as an indirect disinfection method. For example, it can be used for the surface disinfection of silkworm rearing auxiliary facilities, including walls, doors and windows of the sericulture rooms.

Chemical control methods

Chemical control methods mainly use chemical drugs for the disinfection and treatment of pébrine disease. Pharmacological disinfection can result in denaturation of the protein of pathogenic microorganisms, and cause them to lose pathogenicity. In contrast, pharmacological treatment utilizes the specificity of drugs to block the proliferation of *N. bombycis*, thereby achieving the purpose of treating pébrine disease. Disinfectants containing chlorine are currently the longest lasting and most widely used in sericulture production. Both chloroisocyanuric acid and glutaraldehyde methylbromide show good disinfection and sterilization effects. Li *et al*. found that when treated

Table 5 Biosensor and MALDI methods for the detection of *N. bombycis*

Methods	Detection targets	Limit of detection	Detection time	Advantages	Disadvantages	References
C60@Pt-Pd-hemin/G-quadruplex	Spore wall proteins	0.56 pg/mL	50-60min	● Ease of use and rapid ● No cross reaction and time saving ● High sensitivity and specificity ● Without the labeling process of protein enzymes and electronic media	● Complex synthesis of nanoparticles ● Require certain operational skills ● Need electrochemical equipment ● Not suitable for field applications	(Golub *et al.*, 2013; Wang *et al.*, 2014; Wang *et al.*, 2015; Zhou *et al.*, 2013)
ECPA- Fe3O4NPs	Total proteins	0.54 pg/mL	60min	● No cross reaction ● Ease of use and rapid ● Good quantitative ability ● High sensitivity and specificity ● Overcoming the shortcomings of proteases in electrocatalytic signal amplification	● Complex synthesis and modification of nanoparticles ● Require certain operational skills ● Need electrochemical equipment ● Not suitable for field applications	(Ferapontova *et al.*, 2008; Tong *et al.*, 2011; Wang *et al.*, 2016; Yu and Lai, 2013; Zheng *et al.*, 2014)
MALDI-TOF MS	/	/	40min	● Ease of use and rapid ● High sensitivity, specificity, and accuracy ● The ability to distinguish different species	● Require certain operational skills ● Expensive and complex instruments	(Buchan and Ledeboer, 2014; Kajiwara *et al.*, 2016; Kajiwara and Murakami, 2019; Spanu *et al.*, 2012; Zhou *et al.*, 2017)

with the chlorine disinfectant trichloroisocyanuric acid at a concentration of at least 800 mg/L for more than 6 min, the mortality rate of *N. bombycis* was 100% (Li *et al.*, 2023). However, this chemical has a strong irritant effect on silkworms and humans and also is strongly corrosive to metal equipment. The mixed disinfectant of aldehydes and ammonium salts, treated at a concentration of at least 200 mg/L for more than 6 min, can result in a 100% mortality rate of *N. bombycis* (Li *et al.*, 2023). It has low toxicity, little corrosion to metals and can be used as a partial substitute for chlorine-containing disinfectants. The main method of pharmacological treatment is through the use of antifungal and antiparasitic drugs. Some chemical drugs, including carbendazim, lebendazole, and albendazole, can be

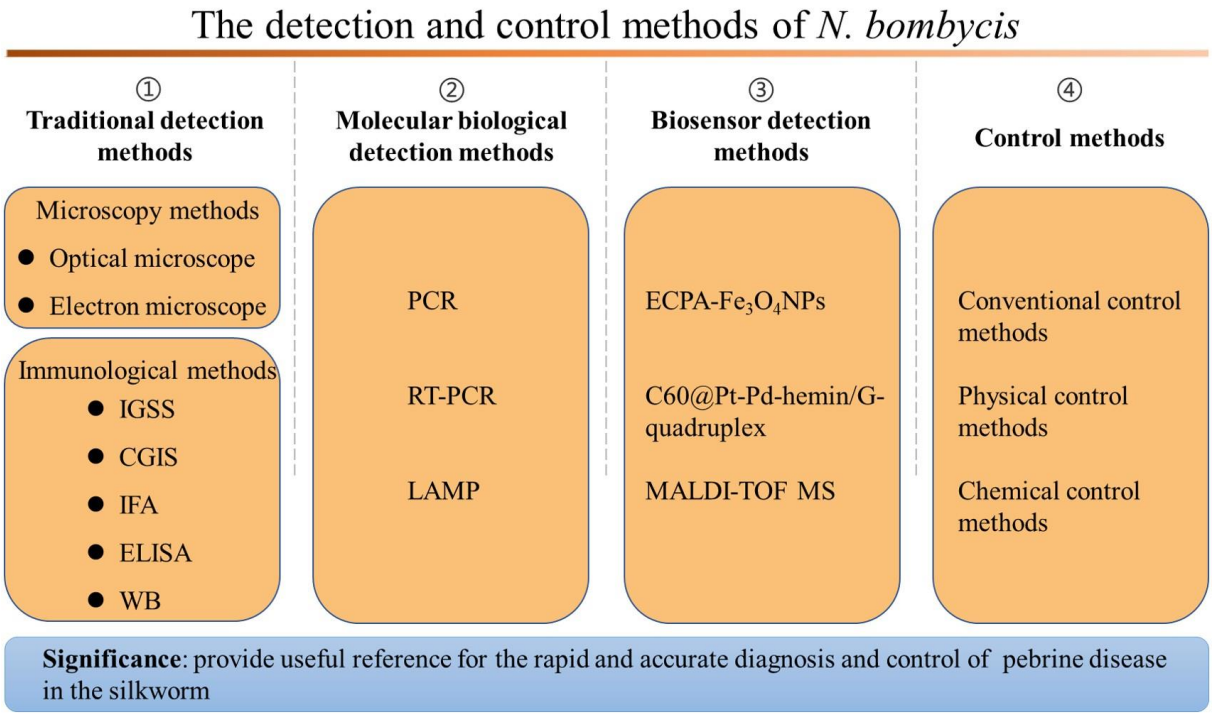

Fig. 2 The detection and control methods of *N. bombycis*

used to treat pébrine disease. Huang et al. showed that albendazole had a good therapeutic effect and low toxicity to silkworms (Huang *et al.*, 2016). Consequently, albendazole can be used to treat pébrine diseases either by soaking mulberry leaves in a solution (1000 mg/L) or directly spraying medicinal powder on mulberry leaves (1 g/kg), together with adding the medicine once a day, which can achieve good therapeutic efficacy (Huang *et al.*, 2016). It can also be blended with cosolvents to form a combinatorial drug containing albendazole, which not only has good therapeutic effects on pébrine disease via peroral infection, but also has the same therapeutic effect on pébrine disease transmitted through silkworm eggs (Huang *et al.*, 2016). The combination drug of albendazole has no toxicity to silkworms and can further enhance their vitality. The discovery and utilization of these therapeutic drugs sufficiently demonstrate their therapeutic prospects for pébrine disease. According to above research, the prevention and control of pébrine disease may also be developed from a single prevention-oriented approach in the past to a comprehensive control system based on prevention and control. Although a variety of chemical drugs have therapeutic effects on pébrine disease, the production and application of these chemical drugs still have significant limitations due to the toxic effects of the drugs themselves, the intracellular parasitic characteristics of *N. bombycis*, their resistance to imidazole drugs and the strong secondary infectivity of microsporiasis in the silkworm bed. Further research is needed to develop composite control agents that have a long retention time on mulberry leaves or silkworms and address a variety of silkworm diseases.

Conclusion and prospect

Pébrine disease causes a decline in cocoon quality and also poses potential food safety issues for silkworms. Since the discovery of *N. bombycis* in silkworms, it has caused huge economic losses to the sericulture countries around the world and is currently listed as the only quarantine object for silkworm egg production. As the sericulture production scale has increased, the occurrence frequency and harmfulness of pébrine disease has also increased. Therefore, the immediate and efficient detection of *N. bombycis* is beneficial for the prevention and control of pébrine disease and also guarantees the healthy development of sericulture production. Efficient detection of pébrine also reduces the safety risk of silkworm food. The detection methods of *N. bombycis* have evolved from visual observation to microscopic examination and then to immunological and molecular biological detection. The cross-combination of technologies have been developed in multiple fields, including electrochemical technology, nanomaterials, and artificial enzymes. New biosensor methods have also been developed for the detection of *N. bombycis* (Figure 2). However, the most mainstream detection method remains the microscopic examination of adult females. Examination using optical microscopy is relatively simple and reliable; but, its sensitivity is very low, and it usually needs to

be observed in a late stage of infection. This often results in a significant lag in detection. Electron microscopy examination must be conducted using ultra-thin sections before observation, which has high requirements for technical equipment and operator training. Therefore, microscopic detection is mainly used for scientific research and is not suitable for on-site diagnosis and identification of pébrine disease. In sericulture production, the detection from adult females and incubated larvae alone cannot completely prevent the occurrence of pébrine disease. Notably, *N. bombycis* should be strictly monitored at all stages of sericulture to prevent infection from external infectious sources, such as mulberry leaves and contaminated breeding materials. This can only be achieved on-site using applicable testing methods. In terms of sensitivity, specificity, detection speed, and reproducibility, the practical value of nucleic acid-based assays is superior to traditional techniques, such as microscopy and immunology. However, PCR technology requires strong experimental skills in operators, and the staining solution used during observation results has significant human toxicity. The sensitivity and specificity of qPCR and LAMP technologies are higher than the PCR method, and their detection results are also quantifiable. However, the main disadvantage is that precise instruments and equipment are needed to monitor the results. The biosensor detection method has high sensitivity and specificity, and is not limited by precision instruments. In addition, newly emerging detection technologies such as FF-LAMP and biosensors have characteristics of high throughput, quantification, and simultaneous analysis of multiple pathogen specimens. However, further research is needed to determine whether the above methods can be applied to real-world production. In summary, although each of the detection methods has its advantages, there are also limitations that make it difficult to be successfully used in production. As a consequence, introducing more detection methods into production and on-site for instant detection in the field remains a challenge. To manage pébrine disease in the sericulture industry and reduce sericulture losses, researchers should develop new testing methods that are highly efficient, portable, easy to operate and suitable for immediate on-site detection. These methods should rapidly detect pébrine disease and allow for immediate control procedures.

Acknowledgements

This work was supported by the Key Technology Research and Development (Modern Agriculture) Program of Zhenjiang City (Grant Number: NY2023007).

All authors read and approved the final article, and this article has not been published and is not under consideration for publication elsewhere. We are thankful to the anonymous reviewers for reviewing our manuscript and providing helpful comments and suggestions. We thank LetPub (www.letpub.com.cn) for its linguistic assistance during the preparation of this manuscript.

References

Adl SM, Simpson AG, Lane CE, Lukeš J, Bass D, Bowser SS*, et al.* The revised classification of eukaryotes. J Eukaryot Microbiol. 59: 429-493, 2012.

Alía A, Andrade MJ, Córdoba JJ, Martín I, Rodríguez A. Development of a multiplex real-time PCR to differentiate the four major Listeria monocytogenes serotypes in isolates from meat processing plants. Food Microbiol. 87: 103367, 2020.

Bagheri M, Zahmatkesh A, Moharrami M, Nematollahian S, Torkaman M. Diagnosis of pebrine disease in silkworm using molecular methods. Arch Razi Inst. 78: 1185-1191, 2023.

Bateman KS, Wiredu-Boakye D, Kerr R, Williams BA, Stentiford GD. Single and multi-gene phylogeny of Hepatospora (Microsporidia) - a generalist pathogen of farmed and wild crustacean hosts. Parasitology. 143: 971-982, 2016.

Bellstedt DU, Pirie MD, Visser JC, de Villiers MJ, Gehrke B. A rapid and inexpensive method for the direct PCR amplification of DNA from plants. Am J Bot. 97: e65-8, 2010.

Bourgeois AL, Rinderer TE, Beaman LD, Danka RG. Genetic detection and quantification of *Nosema apis* and *N. ceranae* in the honey bee. J Invertebr Pathol. 103: 53-58, 2010.

Breaker RR. Tech.Sight. Molecular biology. Making catalytic DNAs. Science. 290: 2095-2096, 2000.

Buchan BW, Ledeboer NA. Emerging technologies for the clinical microbiology laboratory. Clin Microbiol Rev. 27: 783-822, 2014.

Cai S, Lu X, Qiu H, Li M, Feng Z. Identification of a *Nosema bombycis* (Microsporidia) spore wall protein corresponding to spore phagocytosis. Parasitology. 138: 1102-1109, 2011.

Cao C, Lin Y, Lv J, Hua Q. Research progress of diagnosis of *Noesma bombycis*. J Anhui Agri Sci. 44: 1-3, 2016.

Chaimanee V, Pettis JS, Chen Y, Evans JD, Khongphinitbunjong K, Chantawannakul P. Susceptibility of four different honey bee species to *Nosema ceranae*. Vet Parasitol. 193: 260-265, 2013.

Chen J, Guo W, Dang X, Huang Y, Liu F, Meng X*, et al.* Easy labeling of proliferative phase and sporogonic phase of microsporidia *Nosema bombycis* in host cells. PLoS One. 12: e0179618, 2017.

Chen X, Huang K, Shen Z, Wang H, Huang J, Zhuang M*, et al.* Studies on PCR inspection technique of *Nosema bombycis*. Acta Seric Sin. 22: 229-234, 1996.

Cheng B, Jia Y, Wen Y, Hou W, Xu K, Liang C*, et al.* Integrative analysis of microRNA and mRNA sequencing data identifies novel candidate genes and pathways for developmental dysplasia of hip. Cartilage. 13: 1618-1626, 2021.

Choi JR, Hu J, Feng S, Wan Abas WA, Pingguan-Murphy B, Xu F. Sensitive biomolecule detection in lateral flow assay with a portable temperature-humidity control device. Biosens Bioelectron. 79: 98-107, 2016.

Chozas M, Dashti A, Prieto-Pérez L, Pérez-Tanoira R, Cobo E, Bailo B*, et al. Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (microsporidia) in HIV-positive patients in central Spain. Med Mycol. 61, 2023.

Corcoran GD, Tovey DG, Moody AH, Chiodini PL. Detection and identification of gastrointestinal microsporidia using non-invasive techniques. J Clin Pathol. 48: 725-727, 1995.

Dai W, Chen G, Peng X, Li X, Ma L, Tang X*, et al.* Staining and discrimination of *Nosema bombycis* spores with fluorescent brightener 28 and propidium iodide. Acta Seric Sin. 43: 62-67, 2017.

Dai W, Qi J, Chen H, Zhang Z, Shang R, Zhang Y*, et al.* Rapid and sensitive detection of *Nosema bombycis* using loop-mediated isothermal amplification and colorimetric nanogold. ScienceAsia. 45, 2019.

Dang X, Pan G, Li T, Lin L, Ma Q, Geng L*, et al.* Characterization of a subtilisin-like protease with apical localization from microsporidian *Nosema bombycis*. J Invertebr Pathol. 112: 166-174, 2013.

Erler S, Lommatzsch S, Lattorff HM. Comparative analysis of detection limits and specificity of molecular diagnostic markers for three pathogens (Microsporidia, *Nosema* spp.) in the key pollinators *Apis mellifera* and *Bombus terrestris*. Parasitol Res. 110: 1403-1410, 2012.

Esvaran V, Jagadish A, Terenius O, Suraporn S, Mishra RK, Ponnuvel KM. Targeting essential genes of *Nosema* for the diagnosis of pebrine disease in silkworms. Ann Parasitol. 66: 303-310, 2020.

Esvaran VG, Mohanasundaram A, Mahadeva S, Gupta T, Ponnuvel KM. Development and comparison of real-time and conventional PCR tools targeting *β-tubulin* gene for detection of *Nosema* infection in silkworms. J Parasit Dis. 43: 31-38, 2019.

Ferapontova EE, Olsen EM, Gothelf KV. An RNA aptamer-based electrochemical biosensor for detection of theophylline in serum. J Am Chem Soc. 130: 4256-4258, 2008.

Franzen C. Microsporidia: how can they invade other cells? Trends Parasitol. 20: 275-279, 2004.

Franzen C. How do microsporidia invade cells? Folia Parasitol (Praha). 52: 36-40, 2005.

Fu Z, He X, Cai S, Liu H, He X, Li M*, et al.* Quantitative PCR for detection of *Nosema bombycis* in single silkworm eggs and newly hatched larvae. J Microbiol Methods. 120: 72-78, 2016.

Garcia LS. Laboratory identification of the microsporidia. J Clin Microbiol. 40: 1892-1901, 2002.

Golub E, Freeman R, Willner I. Hemin/G-quadruplex-catalyzed aerobic oxidation of thiols to disulfides: application of the process for the development of sensors and aptasensors and for probing acetylcholine esterase activity. Anal Chem. 85: 12126-12133, 2013.

Guo Y, Liu J, Yang G, Sun X, Chen HY, Xu JJ. Multiple turnovers of DNAzyme for amplified detection of ATP and reduced thiol in cell homogenates. Chem Commun (Camb). 51: 862-865, 2015.

Haag KL, James TY, Pombert JF, Larsson R, Schaer TM, Refardt D*, et al.* Evolution of a morphological novelty occurred before genome compaction in a lineage of extreme parasites. Proc Natl Acad Sci U S A. 111: 15480-5, 2014.

Han B, Pan G, Weiss LM. Microsporidiosis in humans. Clin Microbiol Rev. 34: e0001020, 2021.

Han J, Zhuo Y, Chai YQ, Yuan YL, Yuan R. Novel electrochemical catalysis as signal amplified strategy for label-free detection of neuron-specific enolase. Biosens Bioelectron. 31: 399-405, 2012.

Hatakeyama Y, Hayasaka S. A new method of pebrine inspection of silkworm egg using multiprimer PCR. J Invertebr Pathol. 82: 148-151, 2003.

He P, Chen H, Sun J, Wang Q, Tang X, Zhang Y*, et al.* Use of DNA nanosensors based on upconverting nanoparticles for detection of *Nosema bombycis* by fluorescence resonance energy transfer. Folia Microbiol (Praha). 67: 419-425, 2022.

He Y, Wu S, Lu X, Qiu H, Shuai H, Zhang X*, et al.* Influence of DNA extraction methods on detection of *Nosema bombycis* by traditional PCR and real-time PCR methods. Acta Entomol Sin. 54: 1329-1234, 2011.

He Z, Ni Q, Song Y, Wang R, Tang Y, Wu Y*, et al.* Development of a nucleic acid lateral flow strip for rapid, visual detection of *Nosema bombycis* in silkworm eggs. J Invertebr Pathol. 164: 59-65, 2019.

Huang S, Lu X, Wang F, Jin W, Chen S. Comparative study on spore surface proteins of two microsporidia and their infectivity to silkworm *Bombyx mori*. Acta Seric Sin. 30: 157-163, 2004.

Huang WF, Tsai SJ, Lo CF, Soichi Y, Wang CH. The novel organization and complete sequence of the ribosomal RNA gene of *Nosema bombycis*. Fungal Genet Biol. 41: 473-481, 2004.

Huang X, Pan Z, Luo M, Huang S, An C, Mo Y*, et al.* Effect test of ultraviolet irradiation inactivation on silkworm (*Bombyx mori*) disease pathogen Guangxi Seric. 48: 24-26, 2011.

Huang X, Pan Z, Wei T, Tang Q, Jia X, Huang S*, et al.* Screening and application of drugs for pebrine disease of *Bombyx mori*. Guangxi Sericul. 53: 6-11, 2016.

Huang Y, Chen J, Sun B, Zheng R, Li B, Li Z*, et al.* Engineered resistance to *Nosema bombycis* by in vitro expression of a single-chain antibody in Sf9-III cells. PLoS One. 13: e0193065, 2018.

Ignatius R, Henschel S, Liesenfeld O, Mansmann U, Schmidt W, Köppe S*, et al.* Comparative evaluation of modified trichrome and Uvitex 2B stains for detection of low numbers of microsporidial spores in stool specimens. J Clin Microbiol. 35: 2266-2269, 1997.

Ignatius R, Lehmann M, Miksits K, Regnath T, Arvand M, Engelmann E*, et al.* A new acid-fast trichrome stain for simultaneous detection of

Cryptosporidium parvum and microsporidial species in stool specimens. J Clin Microbiol. 35: 446-449, 1997.
Ironside JE. Diversity and recombination of dispersed ribosomal DNA and protein coding genes in microsporidia. PLoS One. 8: e55878, 2013.
Izquierdo F, Moura H, Bornay-Llinares FJ, Sriram R, Hurtado C, Magnet Á*, et al.* Production and characterization of monoclonal antibodies against *Encephalitozoon intestinalis* and *Encephalitozoon* sp. spores and their developmental stages. Parasit Vectors. 10: 560, 2017.
Jagadish A, Khajje D, Tony M, Nilsson A, de Miranda JR, Terenius O*, et al.* Development and optimization of a TaqMan assay for *Nosema bombycis*, causative agent of pebrine disease in *Bombyx mori* silkworm, based on the beta-tubulin gene. J Microbiol Methods. 186: 106238, 2021.
Jansson V, Jansson K. Enzymatic chemiluminescence assay for inorganic pyrophosphate. Anal Biochem. 304: 135-137, 2002.
Jaroenram W, Arunrut N, Kiatpathomchai W. Rapid and sensitive detection of shrimp yellow head virus using loop-mediated isothermal amplification and a colorogenic nanogold hybridization probe. J Virol Methods. 186: 36-42, 2012.
Jiang K, Zhu Y, Liu W, Feng Y, He L, Guan W*, et al.* Development of a loop-mediated isothermal amplification (LAMP) for the detection of F5 fimbriae gene in enterotoxigenic *Escherichia coli* (ETEC). Curr Microbiol. 65: 633-638, 2012.
Joseph J, Murthy S, Garg P, Sharma S. Use of different stains for microscopic evaluation of corneal scrapings for diagnosis of *Microsporidial keratitis*. J Clin Microbiol. 44: 583-585, 2006.
Joseph J, Vemuganti GK, Garg P, Sharma S. Histopathological evaluation of ocular microsporidiosis by different stains. BMC Clin Pathol. 6: 6, 2006.
Kajiwara H, Hinomoto N, Gotoh T. Mass fingerprint analysis of spider mites (Acari) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for rapid discrimination. Rapid Commun Mass Spectrom. 30: 1037-1042, 2016.
Kajiwara H, Murakami R. Rapid detection of silkworm microsporidia by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Invertebr Pathol. 166: 107223, 2019.
Kajiwara H, Murakami R. Rapid detection of silkworm microsporidia by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Journal of invertebrate pathology. 166: 107223, 2019.
Karunakar P, P BS, V K. In silico modelling and virtual screening for identification of inhibitors for spore wall protein-5 in *Nosema bombycis*. J Biomol Struct Dyn. 40: 1748-1763, 2022.
Keeler SP, Ferro PJ, Brown JD, Fang X, El-Attrache J, Poulson R*, et al.* Use of FTA sampling cards for molecular detection of avian influenza virus in wild birds. Avian Dis. 56: 200-207, 2012.
Keeling PJ. Congruent evidence from alpha-tubulin and beta-tubulin gene phylogenies for a zygomycete origin of microsporidia. Fungal Genet Biol. 38: 298-309, 2003.
Keeling PJ, Fast NM. Microsporidia: biology and evolution of highly reduced intracellular parasites. Annu Rev Microbiol. 56: 93-116, 2002.
Keeling PJ, Luker MA, Palmer JD. Evidence from beta-tubulin phylogeny that microsporidia evolved from within the fungi. Mol Biol Evol. 17: 23-31, 2000.
Kiani-Azad K, Bagheri M, Sadeghi M, Nematollahian S, Zahmatkesh A, Moharrami M*, et al.* Molecular characterization of a new *Nosema bombycis* strain detected in iranian silkworm farms. Acta Parasitol. 67: 1364-1371, 2022.
Klee J, Tek Tay W, Paxton RJ. Specific and sensitive detection of *Nosema bombi* (Microsporidia: Nosematidae) in bumble bees (*Bombus* spp.; Hymenoptera: Apidae) by PCR of partial rRNA gene sequences. J Invertebr Pathol. 91: 98-104, 2006.
Kokoskin E, Gyorkos TW, Camus A, Cedilotte L, Purtill T, Ward B. Modified technique for efficient detection of microsporidia. J Clin Microbiol. 32: 1074-1075, 1994.
Lee SC, Corradi N, Byrnes EJ, 3rd, Torres-Martinez S, Dietrich FS, Keeling PJ*, et al.* Microsporidia evolved from ancestral sexual fungi. Curr Biol. 18: 1675-1679, 2008.
Lee SH, Joung M, Yoon S, Choi K, Park WY, Yu JR. Multiplex PCR detection of waterborne intestinal protozoa: microsporidia, Cyclospora, and Cryptosporidium. Korean J Parasitol. 48: 297-301, 2010.
Li L, Su Z, Zhang Y, Shao Y, Bai X. Effects of different disinfection methods on the survival and infectivity of *Nosema bombycis*. J Environ Entomol. 4: 1130-1136, 2023.
Li Y, Liu H, Wu Z, Pan G, Xie L, Wu S*, et al.* Preparation and identification of monoclonal antibodies against *Nosema Bombycis*. Acta Seric Sin. 33: 138-141, 2007.
Li Y, Xie L, Pan G, Wu Z, Pang M, Zhou Z. Establishment and application of immunofluorescence detection method for antibody of *Nosema bombycis*. J Southwest Agric Univ. 28: 990-993, 2006.
Li Z, Wang Y, Wang L, Zhou Z. Molecular and biochemical responses in the midgut of the silkworm, *Bombyx mori*, infected with *Nosema bombycis*. Parasit Vectors. 11: 147, 2018.
Liu H, Pan G, Luo B, Li T, Yang Q, Vossbrinck CR*, et al.* Intraspecific polymorphism of rDNA among five *Nosema bombycis* isolates from different geographic regions in China. J Invertebr Pathol. 113: 63-69, 2013.
Liu H, Pan G, Song S, Xu J, Li T, Deng Y*, et al.* Multiple rDNA units distributed on all chromosomes of *Nosema bombycis*. J Invertebr Pathol. 99: 235-238, 2008.
Liu J, Qian K, Shen ZY. Rapid detection of silkworm microparticle disease by PCR and development of diagnostic kit. Animal quarantine in China. 26: 49-53, 2009.

Liu J, Cao Y, Smith J, Xu X. Studies on the application of PCR molecular diagnosis to silkworms with simulated pebrine disease. Sci Agric Sin. 37: 1925-1931, 2004.

Liu J, Cheng W, Yan Y, Wei J, Yang J. Detection of pebrine disease in *Bombyx mori* eggs with the loop-mediated isothermal amplification (LAMP) method based on *EB1* gene. Acta Entomol Sin. 58: 846-855, 2015.

Liu J, Lu K, Xu X, Yan H, Ning B. Studies on the use of the mono-clonal antibodies immunogold and silver staining technique as a method for diagnosis of *Nosema bombycis* spores. Guangdong Seric. 29: 30-35, 1995.

Liu J, Qian K, Shen Z. Studies on the inspection method for silkworm pebrine by PCR and development of the diagnosis kit. Chin Anim Health Insp. 26: 49-53, 2009.

Liu J, Zeng L. Stain and discrimination of *Nosema bombycis* spores with calcofluor white M2R. Acta Entomol Sin. 50: 1185-1186, 2007.

Long M, Tan Y, Yu B, Pan G, Zhou Z. Expression of *Nosema bombycis* polar tube protein 1 in lepidopteran Sf9 cells and its effect on microsporidian proliferation. J Invertebr Pathol. 172: 107350, 2020.

Maikai BV, Umoh JU, Lawal IA, Kudi AC, Ejembi CL, Xiao L. Molecular characterizations of *Cryptosporidium*, *Giardia*, and *Enterocytozoon* in humans in Kaduna State, Nigeria. Exp Parasitol. 131: 452-456, 2012.

Mani V, Chikkaveeraiah BV, Patel V, Gutkind JS, Rusling JF. Ultrasensitive immunosensor for cancer biomarker proteins using gold nanoparticle film electrodes and multienzyme-particle amplification. ACS Nano. 3: 585-594, 2009.

Menotti J, Cassinat B, Sarfati C, Liguory O, Derouin F, Molina JM. Development of a real-time PCR assay for quantitative detection of *Encephalitozoon intestinalis* DNA. J Clin Microbiol. 41: 1410-1413, 2003.

Mo L, Drancourt M. Monoclonal antibodies for specific detection of *Encephalitozoon cuniculi*. Clin Diagn Lab Immunol. 11: 1060-1063, 2004.

Moharrami M, Bagheri M, Nematollahian S. Detection and characterization of *Nosema bombycis* using TEM and SEM Techniques. Arch Razi Inst. 77: 1473-1480, 2022.

Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother. 15: 62-69, 2009.

Moura H, Da Silva JL, Sodré FC, Brasil P, Wallmo K, Wahlquist S*, et al.* Gram-chromotrope: a new technique that enhances detection of microsporidial spores in clinical samples. J Eukaryot Microbiol. 43: 94-95, 1996.

Nagai MY, Dalboni LC, Cardoso TN, Correia MS, Pinto SAG, Pinto AAG*, et al.* Effects of homeopathic phosphorus on *Encephalitozoon cuniculi*-Infected macrophages in-vitro. Homeopathy. 108: 188-200, 2019.

Nagy A, Vitásková E, Černíková L, Křivda V, Jiřincová H, Sedlák K*, et al.* Evaluation of TaqMan qPCR system integrating two identically labelled hydrolysis probes in single assay. Sci Rep. 7: 41392, 2017.

Nicolas C. Microsporidia: Eukaryotic Intracellular Parasites Shaped by Gene Loss and Horizontal Gene Transfers. Annu Rev Microbiol. 69: 167-183, 2015.

Niu A, Liu H, Zhou M, Zhu F, Fang Z. Detection of microsporidia with chromotrop staining. Chin J Zoonoses. 16: 94, 2000.

Njiru ZK, Mikosza AS, Armstrong T, Enyaru JC, Ndung'u JM, Thompson AR. Loop-mediated isothermal amplification (LAMP) method for rapid detection of *Trypanosoma brucei* rhodesiense. PLoS Negl Trop Dis. 2: e147, 2008.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N*, et al.* Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28: e63, 2000.

Omura M, Furuya K, Kudo S, Sugiura W, Azuma H. Detecting immunoglobulin M antibodies against microsporidian *Encephalitozoon cuniculi* polar tubes in sera from healthy and human immunodeficiency virus-infected persons in Japan. Clin Vaccine Immunol. 14: 168-172, 2007.

Pan Z, Gong C, Zheng X, Xue R, Cao G, Shen W. Study on the detection sensitivity of PCR method for silkworm *Nosema bombycis* DNA. J Chang Inst Technol. 21: 51-54, 2007.

Polley SD, Boadi S, Watson J, Curry A, Chiodini PL. Detection and species identification of microsporidial infections using SYBR Green real-time PCR. J Med Microbiol. 60: 459-466, 2011.

Prasa NR, Govindharaju ST, Keshawa Reddy KS. Positive staining method for detection of pebrine spore *Nosema bombycis* in silkworm *Bombyx mori* L. Sericologia. 40: 575-580, 2000.

Ravikumar G, Raje Urs S, Vijaya Prakash NB, Rao CG, Vardhana KV. Development of a multiplex polymerase chain reaction for the simultaneous detection of microsporidians, nucleopolyhedrovirus, and densovirus infecting silkworms. J Invertebr Pathol. 107: 193-197, 2011.

Refardt D, Ebert D. Quantitative PCR to detect, discriminate and quantify intracellular parasites in their host: an example from three microsporidians in Daphnia. Parasitology. 133: 11-18, 2006.

Salleh FM, Al-Mekhlafi AM, Nordin A, Yasin M, Al-Mekhlafi HM, Moktar N. Evaluation of gram-chromotrope kinyoun staining technique: its effectiveness in detecting microsporidial spores in fecal specimens. Diagn Microbiol Infect Dis. 69: 82-85, 2011.

Salm E, Zhong Y, Reddy B, Jr., Duarte-Guevara C, Swaminathan V, Liu YS*, et al.* Electrical detection of nucleic acid amplification using an on-chip quasi-reference electrode and a PVC REFET. Anal Chem. 86: 6968-6975, 2014.

Sanders JL, Kent ML. Development of a sensitive assay for the detection of *Pseudoloma neurophilia* in laboratory populations of the zebrafish *Danio rerio*. Dis Aquat Organ. 96: 145-156, 2011.

Sauer S, Freiwald A, Maier T, Kube M, Reinhardt R, Kostrzewa M*, et al.* Classification and

identification of bacteria by mass spectrometry and computational analysis. PLoS One. 3: e2843, 2008.

Schwartz DA, Bryan RT, Visvesvara GS. Diagnostic approaches for *Encephalitozoon* infections in patients with AIDS. J Eukaryot Microbiol. 41: 59-60, 1994.

Sheoran AS, Feng X, Singh I, Chapman-Bonofiglio S, Kitaka S, Hanawalt J*, et al.* Monoclonal antibodies against *Enterocytozoon bieneusi* of human origin. Clin Diagn Lab Immunol. 12: 1109-1113, 2005.

Singh P, Mirdha BR, Ahuja V, Singh S. Loop-mediated isothermal amplification (LAMP) assay for rapid detection of Entamoeba histolytica in amoebic liver abscess. World J Microbiol Biotechnol. 29: 27-32, 2013.

Sivaprasad V, Renu S, Nataraju B, Datta RK. Protein A linked latex antisera test for detection of infectious flacherie of silkworm, *Bombyx mori* L. caused by B. mori infectious flacherie virus. Indian J Exp Biol. 35: 1203-1207, 1997.

Sivaprasad V, Satish L, Mallikarjuna G, Chandrakanth N, Josepha AM, Moorthy S. A Field-Friendly Loop-Mediated Isothermal Amplification (FF-LAMP) method for rapid detection of *Nosema bombycis* in silkworm, *Bombyx mori*. Invertebrate Survival Journal. 66-74, 2021.

Spanu T, Posteraro B, Fiori B, D'Inzeo T, Campoli S, Ruggeri A*, et al.* Direct maldi-tof mass spectrometry assay of blood culture broths for rapid identification of Candida species causing bloodstream infections: an observational study in two large microbiology laboratories. J Clin Microbiol. 50: 176-179, 2012.

Stine SW, Vladich FD, Pepper IL, Gerba CP. Development of a method for the concentration and recovery of microsporidia from tap water. J Environ Sci Health A Tox Hazard Subst Environ Eng. 40: 913-925, 2005.

Subrungruang I, Mungthin M, Chavalitshewinkoon-Petmitr P, Rangsin R, Naaglor T, Leelayoova S. Evaluation of DNA extraction and PCR methods for detection of *Enterocytozoon bienuesi* in stool specimens. J Clin Microbiol. 42: 3490-3494, 2004.

Suebsing R, Prombun P, Srisala J, Kiatpathomchai W. Loop-mediated isothermal amplification combined with colorimetric nanogold for detection of the microsporidian *Enterocytozoon hepatopenaei* in penaeid shrimp. J Appl Microbiol. 114: 1254-1263, 2013.

Sun W, Qin P, Gao H, Li G, Jiao K. Electrochemical DNA biosensor based on chitosan/nano-V2O5/MWCNTs composite film modified carbon ionic liquid electrode and its application to the LAMP product of *Yersinia enterocolitica* gene sequence. Biosens Bioelectron. 25: 1264-1270, 2010.

Tajadini M, Panjehpour M, Javanmard SH. Comparison of SYBR green and TaqMan methods in quantitative real-time polymerase chain reaction analysis of four adenosine receptor subtypes. Adv Biomed Res. 3: 85, 2014.

Tang S, Zhang Z, Li Y, He J. A fast stains method for diagnosis of *Nosema bombycis* spores. Chin Seric. 23: 27-28, 2002.

Taniuchi M, Verweij JJ, Sethabutr O, Bodhidatta L, Garcia L, Maro A*, et al.* Multiplex polymerase chain reaction method to detect Cyclospora, Cystoisospora, and Microsporidia in stool samples. Diagn Microbiol Infect Dis. 71: 386-390, 2011.

Techathuvanan C, Draughon FA, D'Souza DH. Loop-mediated isothermal amplification (LAMP) for the rapid and sensitive detection of *Salmonella typhimurium* from pork. J Food Sci. 75: 165-172, 2010.

Tong P, Zhang L, Xu JJ, Chen HY. Simply amplified electrochemical aptasensor of ochratoxin A based on exonuclease-catalyzed target recycling. Biosens Bioelectron. 29: 97-101, 2011.

Toumazou C, Shepherd LM, Reed SC, Chen GI, Patel A, Garner DM*, et al.* Simultaneous DNA amplification and detection using a pH-sensing semiconductor system. Nat Methods. 10: 641-646, 2013.

Verweij JJ, Ten Hove R, Brienen EA, van Lieshout L. Multiplex detection of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in fecal samples using real-time PCR. Diagn Microbiol Infect Dis. 57: 163-167, 2007.

Visvesvara GS, Leitch GJ, da Silva AJ, Croppo GP, Moura H, Wallace S*, et al.* Polyclonal and monoclonal antibody and PCR-amplified small-subunit rRNA identification of a microsporidian, *Encephalitozoon hellem*, isolated from an AIDS patient with disseminated infection. J Clin Microbiol. 32: 2760-2768, 1994.

Wan M, He Y, Zhang H, Zhang F, Qian Y, Lu X. Studies on the method of McAb-ELISA detection of pebrine of *bombyx mori* egg. Bull Seric. 39: 13-16, 2008.

Wang HH, Yin C, Gao J, Tao R, Wang CY, Li YX*, et al.* Development of a real-time TaqMan PCR method for absolute quantification of the biocontrol agent *Esteya vermicola*. Plant Dis. 104: 1694-1700, 2020.

Wang Q, Gan X, Zang R, Chai Y, Yuan Y, Yuan R. An amplified electrochemical proximity immunoassay for the total protein of *Nosema bombycis* based on the catalytic activity of Fe_3O_4NPs towards methylene blue. Biosens Bioelectron. 81: 382-387, 2016.

Wang Q, Song Y, Chai Y, Pan G, Li T, Yuan Y*, et al.* Electrochemical immunosensor for detecting the spore wall protein of *Nosema bombycis* based on the amplification of hemin/G-quadruplex DNAzyme concatamers functionalized Pt@Pd nanowires. Biosens Bioelectron. 60: 118-123, 2014.

Wang Q, Song Y, Xie H, Chai Y, Yuan Y, Yuan R. L-cysteine induced hemin/G-quadruplex concatemers electrocatalytic amplification with Pt-Pd supported on fullerene as a nanocarrier for sensing the spore wall protein of *Nosema bombycis*. Chem Commun (Camb). 51: 1255-1258, 2015.

Wang Z, Ma L. Gold nanoparticle probes. Coord Chem Rev. 253: 1607-18, 2009.

Willner I, Shlyahovsky B, Zayats M, Willner B. DNAzymes for sensing, nanobiotechnology and logic gate applications. Chem Soc Rev. 37: 1153-1165, 2008.

Wolk DM, Schneider SK, Wengenack NL, Sloan LM, Rosenblatt JE. Real-time PCR method for detection of *Encephalitozoon intestinalis* from stool specimens. J Clin Microbiol. 40: 3922-3928, 2002.

Wu S, He YQ, Lu XM, Zhang XF, Shuai JB, Yu HZ*, et al.* Early and simultaneous detection of*Nosema bombycis* (Microsporidia: Nosematidae), nucleopolyhedrovirus (Baculoviridae), and densovirus (Parvoviridae) by multiplex real-time polymerase chain reaction in *Bombyx mori* (Lepidoptera: Bombycidae). The Canadian Entomologist. 149: 265-275, 2017.

Xie S, Tang Y, Tang D. Converting pyrophosphate generated during loop mediated isothermal amplification to ATP: Application to electrochemical detection of *Nosema bombycis* genomic DNA PTP1. Biosens Bioelectron. 102: 518-524, 2018.

Xie S, Yuan Y, Song Y, Zhuo Y, Li T, Chai Y*, et al.* Using the ubiquitous pH meter combined with a loop mediated isothermal amplification method for facile and sensitive detection of *Nosema bombycis* genomic DNA PTP1. Chem Commun (Camb). 50: 15932-15935, 2014.

Xie S, Zhang J, Yuan Y, Chai Y, Yuan R. An electrochemical peptide cleavage-based biosensor for prostate specific antigen detection via host-guest interaction between ferrocene and β-cyclodextrin. Chem Commun (Camb). 51: 3387-3390, 2015.

Xing D, Xiao Y, Liao S, Li Q, Ye M, Yang Q. Effect of high temperature instant acid treatment to silkworm eggs on blocking transovarial transmission of pebrine disease. Acta Seric Sin. 44: 100-104, 2018.

Yan W, Shen Z, Tang X, Xu L, Li Q, Yue Y*, et al.* Detection of *Nosema bombycis* by FTA cards and loop-mediated isothermal amplification (LAMP). Curr Microbiol. 69: 532-540, 2014.

Yu ZG, Lai RY. Effect of signaling probe conformation on sensor performance of a displacement-based electrochemical DNA sensor. Anal Chem. 85: 3340-3346, 2013.

Zhang F, Wu J, Wang R, Wang L, Ying Y. Portable pH-inspired electrochemical detection of DNA amplification. Chem Commun (Camb). 50: 8416-8419, 2014.

Zhao Q, Zhang Z, Tang Y. A new conjugated polymer-based combination probe for ATP detection using a multisite-binding and FRET strategy. Chem Commun (Camb). 53: 9414-9417, 2017.

Zheng T, Zhang Q, Feng S, Zhu JJ, Wang Q, Wang H. Robust nonenzymatic hybrid nanoelectrocatalysts for signal amplification toward ultrasensitive electrochemical cytosensing. J Am Chem Soc. 136: 2288-2291, 2014.

Zhou J, Zhuang J, Tang J, Li Q, Tang D, Chen G. Dual-nanogold-linked bio-barcodes with superstructures for in situ amplified electronic detection of low-abundance proteins. Mol Biosyst. 9: 622-625, 2013.

Zhou M, Yang Q, Kudinha T, Sun L, Zhang R, Liu C*, et al.* An improved in-house MALDI-TOF MS protocol for direct cost-effective identification of pathogens from blood cultures. Front Microbiol. 8: 1824, 2017.

Zhou Z, Pan G, Xiang Z. A retrospect on researches in *Nosema bombycis* during the past ten years. Sci Seric. 40, 2014.