Trichomonas

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Abstract

The most widely known trichomonad in veterinary medicine is *Tritrichomonas foetus.* It is the etiologic agent of bovine tritrichomonosis, a sexually transmitted disease in extensively managed herds throughout many geographic regions worldwide. The same trichomonad species is also regarded as the causative agent of chronic diarrhea in the domestic cat, although more recent studies observed molecular differences between bovine- and feline-derived *T. foetus*. Trichomonosis in cats has a worldwide distribution and is mainly present among cats from high-density housing environments. Other trichomonads are found as inhabitants of the gastrointestinal tract in birds, such as *Trichomonas gallinae*. Particularly, Columbiformes, Falconiformes, Strigiformes, and wild Passeriformes can be severely affected by avian trichomonads. Diagnosis of trichomonosis is often complicated by the fragility of the parasite. To ensure valid test results, it is essential to collect and handle specimens in the right way prior to analysis. Cultivation tests, the specific amplification of parasites, or a combination of both test methods is the most efficient and most commonly used way to diagnose trichomonosis in animals. Bovine tritrichomonosis is mainly controlled by the identification and withdrawal of infected animals from bovine herds. The control of feline and avian trichomonosis relies mainly on preventive measures.

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14.1 Morphology, Life Cycle, and Host-Pathogen Interactions

In veterinary medicine the most widely known trichomonad is *Tritrichomonas foetus.* It is located in the urogenital tract of cattle and considered the etiologic agent of bovine tritrichomonosis, a sexually transmitted disease throughout many geographic regions worldwide (Bondurant 2005; Ondrak 2016). The same trichomonad species was initially described in 1999 and finally confirmed in 2003 to be the causative agent of chronic diarrhea in the domestic cat (Gookin et al. 1999; Levy et al. 2003). Trichomonosis in cats has a worldwide distribution and is mainly present among cats from high-density housing environments such as catteries, shelters, or breeding facilities (Yao and Köster 2015). Other trichomonads in animals, for example, *Tritrichomonas suis* in swine—presumably genetically identical to *T. foetus* of bovine origin—are commensals and rarely involved in disease. *T. suis* was once thought to cause atrophic rhinitis in pigs, further studies were not able to establish a causal relationship, and *T. suis* is now considered a harmless nasal and gastrointestinal commensal in swine (BonDurant and Honigberg 1994). Isolates of *T. suis* that reside in the stomach, caecum, and nasal cavity of pigs are not of clinical significance to their porcine hosts (Fitzgerald et al. 1958; Hibler et al. 1960; Pakandl 1994; Mostegl et al. 2011).

Trichomonads are occasionally observed in the feces from dogs with diarrhea (Gookin et al. 2005). The parasite was also detected by culture in the feces of 17.2% of puppies from French breeding kennels, which indicates that *T. foetus* may be a common parasite in dogs (Grellet et al. 2010). However, molecular identity of the enteric trichomonads observed in these dogs was not investigated, and the relevance for dogs is not clear. More studies are required to determine the prevalence and clinical significance of *T. foetus* infection in dogs, since the finding could be also attributed to opportunistic overgrowth of the commensal, *Pentatrichomonas hominis*.

In human medicine, the most studied and relevant is *Trichomonas vaginalis* that affects over 150 million people worldwide and is the most common non-viral sexually transmitted disease (Van der Pol 2007).

Other trichomonads are found as inhabitants of the gastrointestinal tract in birds such as *Tetratrichomonas gallinarum* and *Trichomonas gallinae*. Particularly, Columbiformes, Falconiformes, Strigiformes, and wild Passeriformes can be severely affected by avian trichomonads, whereas the majority of infections in Galliformes and Anatiformes are subclinical although severe infections are occasionally reported (Amin et al. 2014).

Other examples of trichomonads found as inhabitants of the gastrointestinal tract are *Trichomonas muris* of mice and *Pentatrichomonas hominis* of a variety of vertebrate species (BonDurant and Honigberg 1994).

14.1.1 Morphology

Trichomonads are taxonomically framed in the Parabasalia class and Trichomonadida order. This order includes protists with a parabasal apparatus and three to five anterior kinetosomes and one posterior kinetosome. They usually bear flagella and have

a conspicuous pelta-axostyle complex, and the recurrent flagella are often associated with a lamellar undulating membrane underlain by a striated costal fiber (Adl et al. 2005). The number of free flagella characterizes each genus of the family Trichomonadidae. Thus, the genus *Tritrichomonas* is characterized by having three free flagella, whereas the genera *Tetratrichomonas* and *Pentatrichomonas* possess four and five flagella, respectively. Among the various species of trichomonads thus far identified, only a number of them are regarded as pathogens (BonDurant and Honigberg 1994).

14.1.1.1 *Tritrichomonas foetus*

Bovine *T. foetus* isolated from the urogenital tract of cattle and feline isolates found in the gastrointestinal tract of the domestic cat are morphologically indistinguishable. However, there appears to be no association between *T. foetus* infection in cats and reported exposure to cattle (Gookin et al. 2004). There is an ongoing debate whether *T. foetus* from cattle and cats should be placed into separate species. A molecular separation of feline and bovine isolates of *T. foetus* based on a number of gene loci—summarized by Yao and Köster (2015)—seems to be possible, but on the transcriptomic level, a separation remains difficult (Reinmann et al. 2012; Slapeta et al. 2010, 2012; Sun et al. 2012; Morin-Adeline et al. 2014, 2015b). There is evidence that *T. foetus* isolates from cats and cattle show differences in pH tolerance (Sect. 14.2.2), and *T. foetus* from cats are able to survive a passage through the alimentary tract of slugs (Morin-Adeline et al. 2015a; Van der Saag et al. 2011). Thus, some authors believe that feline *T. foetus* represents a different species, and a change in name—to *T. blagburni*—has been proposed (Walden et al. 2013). It has been hypothesized that feline *T. foetus* has extended its host range into the bovine reproductive tract (Morin-Adeline et al. 2015a). The picture becomes even more complicated because *T. foetus* isolated from cattle seems to be morphologically and genetically identical to *Tritrichomonas suis*, that is, a commensal observed in the nasal cavity, stomach, cecum, and colon of the domestic pig (Felleisen 1998; Hampl et al. 2001; Tachezy et al. 2002; Reinmann et al. 2012; Slapeta et al. 2012; Sun et al. 2012). Consequently, it was assumed that *T. suis* and *T. foetus* belong to the same species (Tachezy et al. 2002; Lun et al. 2005; Frey and Müller 2012; Yao and Köster 2015). However, more recent epidemiological studies suggest that cross-species transmission from pigs to cattle on the same farm—e.g., by exposure to *T. foetus*-contaminated pig feces—is unlikely to occur (Mueller et al. 2015).

T. foetus has a trophozoite stage, with a pyriform or ovoid appearance, and a size ranging from 8 to 18 μm in length and 4 to 9 μm in width (BonDurant and Honigberg 1994) (Fig. 14.1). The trophozoite has several structures with locomotor function such as flagella and the undulating membrane. The flagella originate from the basal bodies or kinetosomes—located in the apical pole of the cell. Three of the flagella are of similar length to each other and are directed forward, while the fourth flagellum, called the recurrent flagellum, is directed toward the posterior part of the body, associated with it by an undulating membrane, and continues as a free flagellum beyond the posterior end of the undulating membrane (Taylor et al. 1994; Benchimol 2004).

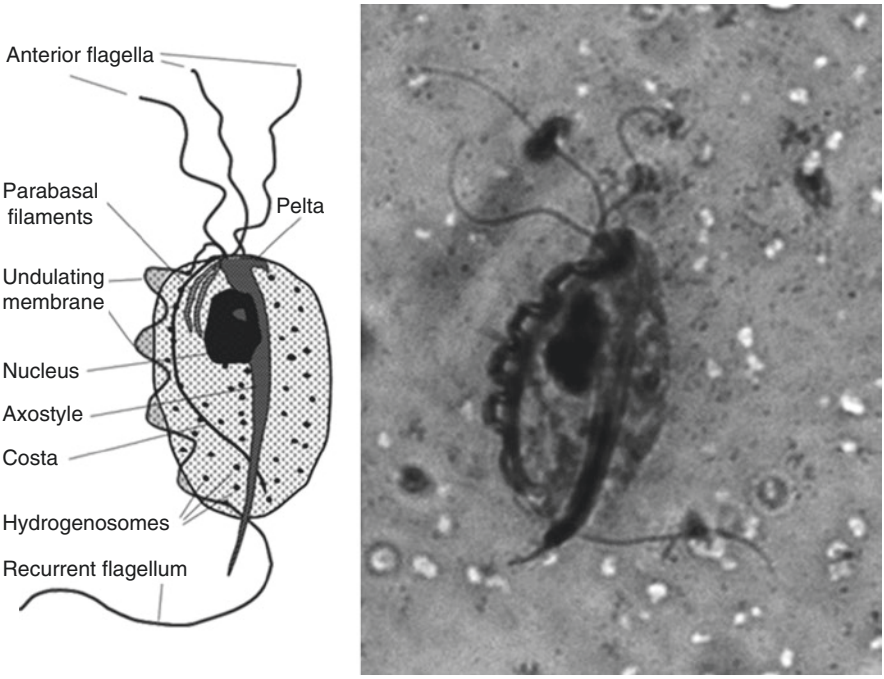

Fig. 14.1 *Tritrichomonas foetus* trophozoites (size, 8–18 × 4–9 μm): schematic and microscopic representation

The internal organelles of *T. foetus* are similar to those of other trichomonads. The cytoplasm contains a series of support elements, including the pelta-axostyle complex, the costal fiber bordering the recurrent flagellum, and the parabasal apparatus. These elements together with the flagellum make up the cytoskeleton (Benchimol 2005). The axostyle originates in the same area of the birth of the flagellum—surrounded at this point by a chromatin ring—and is directed toward the back of the parasite, making prominence at the posterior end. The pelta is a semilunar structure, very little developed, located in the anterior part of the axostyle. Both structures form the pelta-axostyle complex—composed of groups of connected microtubules—forming a kind of slit that houses the nucleus and parabasal bodies (Benchimol 2004, 2005). In *T. foetus*, the axostyle has two functions; it serves as a support organelle and participates in the processes of cell division, allowing the constriction of the nuclei during the karyokinesis (Ribeiro et al. 2002). The costa is a rigid structure that sits on the inner margin of the undulating membrane and serves as a support. The parabasal filaments are also filaments striped perpendicularly, and their mission seems to support the parabasal body—i.e., the Golgi complex. The parabasal filaments and the parabasal body constitute the parabasal apparatus, located in the anterior part of the cell (Benchimol 2004, 2005). *T. foetus* has a simple anterior nucleus and hydrogenosomes, which appear as electro-dense corpuscles that act as functional substitutes for mitochondria. Other cellular components that

can be observed in the cytoplasm are free ribosomes, polysomes, glycogen granules, vesicles, and vacuoles related to processes of endocytosis, digestion, and transport (Benchimol 2004, 2005). Under unfavorable conditions, such as a low concentration of nutrients, the presence of certain drugs such as griseofulvin, or abrupt changes in temperature, trophozoites internalize the flagellum and acquire a form of pseudocyst, which is not surrounded by a manifest cell wall (Pereira-Neves and Benchimol 2009).

As regards nutrition, trichomonads lack a cytostome; they are able to capture food through the cell surface by means of pinocytosis and phagocytosis, with the resulting formation of food vacuoles of different size. Like other trichomonads that inhabit body cavities, *T. foetus* feeds mainly on bacteria, whose proliferation depends on the environment conditions where the parasite is based (Petrin et al. 1998). From the metabolic point of view, *T. foetus* is unable to de novo synthesize purine and pyrimidine nucleotides, as well as complex phosphoglycerides or cholesterol. The parasite obtains its energy through the anaerobic catabolism of carbohydrates, although the trichomonads are able to survive in the presence of oxygen. As already noted, trichomonads lack mitochondria but possess hydrogenosomes that produce molecular hydrogen in anaerobiosis and reduce oxygen. In this way, the parasite manages to keep the pH of its environment close to neutrality favoring its own development (Kleydman et al. 2004).

The reproduction of *T. foetus*—like that of all trichomonads—is asexual (Petrin et al. 1998). The parasite divides by longitudinal binary fission in which the nuclear membrane persists—a type of mitosis referred to as cryptopleuromitosis. In addition, when compared to the trophozoite form, pseudocysts present a different mitosis model, since they first divide the nuclei without dividing their cytoplasm, leading to the formation of multinucleated polymastigotes that persist if the cells are maintained under conditions of stress. When the environmental conditions are again favorable, flagella are externalized, and the new flagellated trophozoites emerge from the multinucleated cells (Pereira-Neves and Benchimol 2009).

14.1.1.2 *Trichomonas gallinae* and *Tetratrichomonas gallinarum*

T. gallinae is the only trichomonad species with a clear pathogenic potential for birds (BonDurant and Honigberg 1994; Amin et al. 2014). *T. gallinarum* is commonly found in the large intestine of gallinaceous and anseriform birds, yet its role in causing disease either in naturally infected chickens and turkeys or via experimental infection is under discussion (Amin et al. 2014). *T. gallinae* trophozoites have an ovoidal to pyriform shape with a size of about 7–11 μm. They are provided with four free anterior flagella and a fifth recurrent one, which does not become free at the posterior pole as it extends for only two-thirds of the body length (Tasca and De Carli 2003; Mehlhorn et al. 2009). Trophozoites of *T. gallinarum* appear mostly pear shaped and range in size from 6 to 15 μm (Clark et al. 2003). They also have four free anterior flagella and a fifth recurrent one, which becomes free at the posterior pole. Another difference to *T. gallinae* is the occurrence of a sphere of lacunes of the endoplasmic reticulum surrounding in a regular distance the nucleus with its

typical perinuclear membranes. Furthermore, the food vacuoles appear to be very large (Mehlhorn et al. 2009).

14.1.2 Life Cycle

14.1.2.1 *Tritrichomonas foetus* in Cattle

Bovine *T. foetus* is located in the genital tract of its natural hosts, *Bos taurus taurus* and *Bos taurus indicus* cattle (Skirrow and BonDurant 1988; Bondurant 2005; Sager et al. 2007). The preferred location of the parasite in the bull is the preputial cavity—concentrating mainly in the penile mucosa and adjacent areas of the posterior preputial mucosa—specifically on the surface of the stratified squamous epithelium of the penis and the proximal foreskin in the fornix area (Clark et al. 1974; Parsonson et al. 1974; Parker et al. 1999). This epithelium undergoes numerous folds—resulting in a greater development of crypts—where *T. foetus* can develop properly by providing a suitable microenvironment for facultative or microaerophilic anaerobic microorganisms (Rhyan et al. 1999). Infection may persist for the life of the bull, in spite of the presence of a measurable humoral immune response in the preputial cavity (Rhyan et al. 1999; Campero et al. 1990; Flower et al. 1983).

In the female, once the infection has occurred, the parasite colonizes the surface of the entire genital system—vagina, cervix, endometrium, and oviduct—in a period of 2 weeks (Parsonson et al. 1976). As observed in natural infections, the parasite is preferentially concentrated in the folds of the cervix (BonDurant 1997). The infection is self-limiting, and the parasite disappears simultaneously from all areas of the genital tract after a period of at least 90–95 days (Parsonson et al. 1976; Rae et al. 2004; Bondurant 2005). In experimental infections of nonpregnant heifers, *T. foetus* infection is typically cleared from the uterus and vagina between weeks 6 and 12 following infection (Parsonson et al. 1976; Anderson et al. 1996; BonDurant et al. 1993; Skirrow and BonDurant 1990a). A very small proportion of cows in infected herds—a fraction less than 1%—have been shown to remain infected throughout pregnancy and into the following breeding season. Fortunately, such carrier cows are rare (Bondurant 2005).

Under natural conditions, *T. foetus* is transmitted directly from an infected animal to a healthy animal, almost exclusively through natural mating (Bondurant 2005). The bulls become infected during the mating of infected cows, remaining asymptomatic carriers (Fig. 14.2). Very rarely, however, the parasite can be transmitted by other routes, for example, mechanically during the practice of artificial insemination or vaginal examination, if contaminated material is used—e.g., using the same glass rod or insemination pipette for different cows or not properly disinfected specula (Murname 1959; Goodger and Skirrow 1986). Mechanical transmission seems to be possible through a healthy bull—i.e., from an infected cow to a receptive cow—if the time between two services does not exceed 20 min (Clark et al. 1977; Goodger and Skirrow 1986; Bondurant 2005; Ondrak 2016).

T. foetus was shown to be able to survive in cryopreserved semen and may be present in semen if it is contaminated with preputial fluid during manual collection

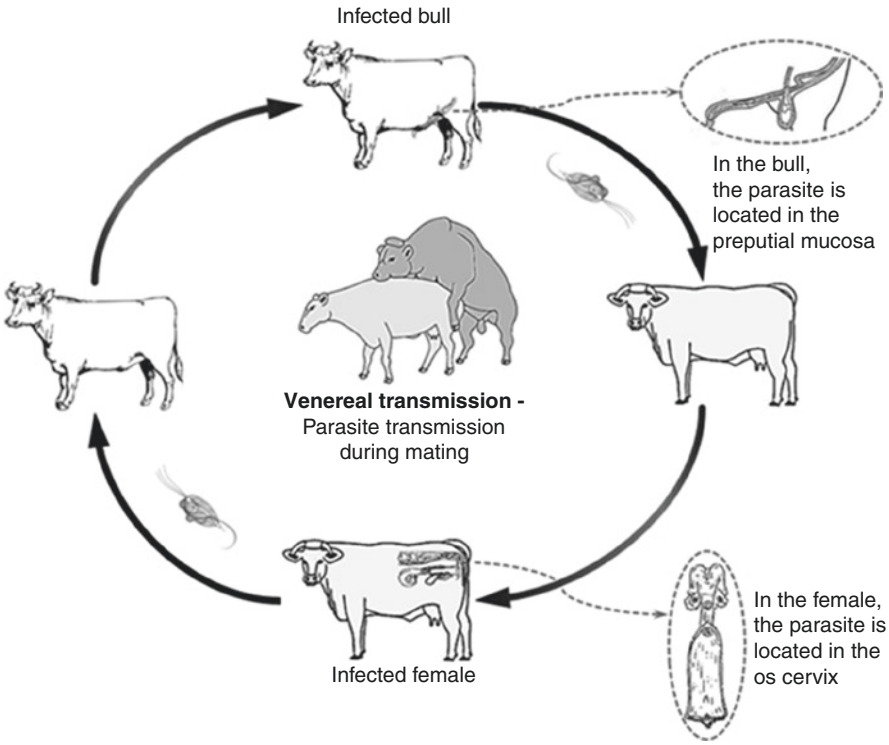

Fig. 14.2 Life cycle of bovine *Tritrichomonas foetus* (David Arranz Solís from SALUVET-UCM is acknowledged for providing this graph)

(Blackshaw and Beattie 1955). Given the resistance of this parasite in fresh, pure, or diluted semen, refrigerated and even cryopreserved, there is the possibility of transmission through artificial insemination with contaminated semen (Bondurant 2005).

Because bulls tend to mount each other, feces in the preputial cavity is commonly found. This fecal material may contain non-*T. foetus* trichomonads, such as *Pentatrichomonas hominis* and any number of *Tetratrichomonas* species that have been shown to be nonpathogenic (Taylor et al. 1994; Campero et al. 2003; Hayes et al. 2003). The opportunity for transmission of *T. foetus* between males is regarded as very limited.

14.1.2.2 *Tritrichomonas foetus* in Cats

Feline *T. foetus* appears to be host adapted, i.e., adapted to the intestinal tract of cats. After experimental orogastric infection of kittens, feline *T. foetus* has been demonstrated to colonize the lumen of the ileum, caecum, colon, and rectum 203 days after infection (Gookin et al. 2001). In naturally infected cats, massive numbers of trichomonads can be observed at the surface of the colonic epithelium and within the colonic crypts (Yaeger and Gookin 2005). Although the presence of *T. foetus* in the uterus of a cat with pyometra has been described, it has been speculated that the

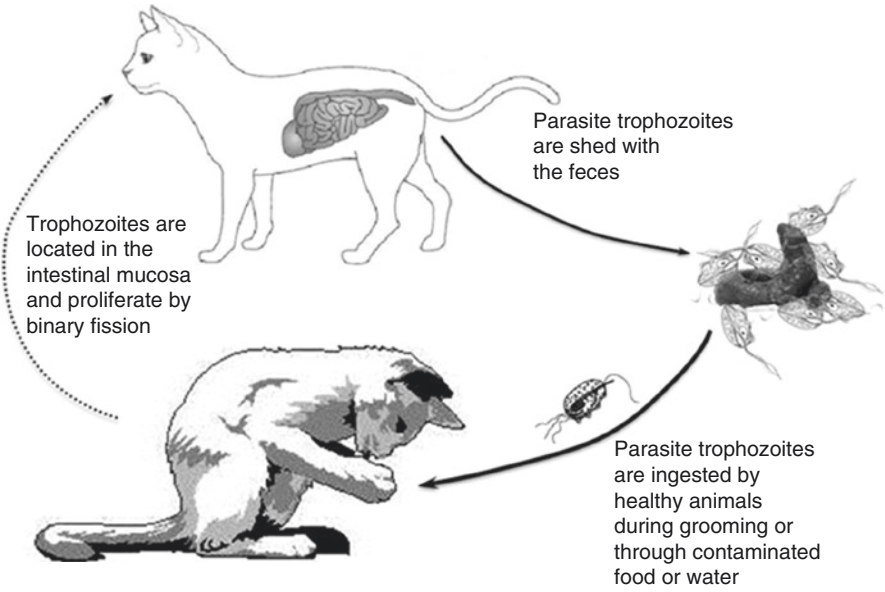

Fig. 14.3 Life cycle of feline *Tritrichomonas foetus* (David Arranz Solís from SALUVET-UCM is acknowledged for providing this graph)

parasite could have accidentally accessed the genital area through contact with contaminated feces (Dahlgren et al. 2007). However, colonization of the reproductive tract in both male and female cats from breeding grounds with a high prevalence of feline tritrichomonosis has not been observed (Gray et al. 2010). Feline *T. foetus* infection occurs by direct fecal-oral transmission. Infected cats are shedding trophozoites with their feces, and transmission occurs when two or more cats share the same litter box (Fig. 14.3). Trophozoites would adhere to the hair of the animals and could be ingested during grooming (Gookin et al. 2004; Tolbert and Gookin 2009). The viability of the parasite in the environment is limited though it can withstand several days at room temperature facilitating its transmission (Hale et al. 2009). *T. foetus*-contaminated food and less likely water may be also a relevant route for transmission. Further, shedding of viable *T. foetus* has been demonstrated in some slug species, which were fed cat food spiked with trophozoites of a feline *T. foetus* isolate, suggesting that invertebrates like slugs could play a role as mechanical vectors (Van der Saag et al. 2011).

14.1.2.3 *Trichomonas gallinae* and *Tetratrichomonas gallinarum*

The rock pigeon—*Columba livia*—was regarded as the primary host of *T. gallinae* and has been considered responsible for the worldwide distribution of this protozoal infection (Stabler 1954; Harmon et al. 1987). Other species within the Columbiformes, Falconiformes, Strigiformes, and, most recently, different Passeriformes have been recognized as potential hosts (Forrester and Foster 2008; Robinson et al. 2010). However, only a few natural occurrences of trichomonosis have been reported in

gallinaceous birds like turkeys and chickens (Levine and Brandly 1939). The preferred site for *T. gallinae* is the upper digestive tract including the mouth, pharynx, esophagus, and crop, with the parasite rarely found posterior to the proventriculus (Cauthen 1936). Transmission by direct contact seems to be the most efficient route to establish an infection—e.g., via the crop milk from infected parent birds to the nestlings during feeding (Stabler 1954). In adult pigeons, the infection can occur during courtship while raptors can be infected from prey animals carrying the parasite. The infection of turkeys and chickens happens mainly via drinking water contaminated by pigeons (BonDurant and Honigberg 1994). *Trichomonas gallinae* is unable to form true cysts, even though cyst-like stages—pseudocysts—have been reported (Tasca and De Carli 2003; Mehlhorn et al. 2009). These pseudocysts may provide another route of transmission and an environmentally resistant stage during unfavorable conditions.

Tetratrichomonas gallinarum locates in the intestinal tract of different poultry species including chickens, turkeys, guinea fowl, quails, ducks, and geese and can be transmitted via consumption of contaminated food (BonDurant and Honigberg 1994). Pseudocysts of *T. gallinarum* have been reported in vivo and in vitro possibly protecting the parasite during fecal-oral transmission (Mehlhorn et al. 2009).

14.1.3 Host-Pathogen Interactions

14.1.3.1 *T. foetus* Infection in Cattle

Once infected, the male acts as an asymptomatic carrier throughout his life (Clark et al. 1974; Parsonson et al. 1974; Parker et al. 1999). Minor histological changes are observed with increased accumulations of neutrophils followed by an infiltrate of lymphocytes and plasma cells penetrating into the intraepithelial area and coalescing in the subepithelium to form lymphoid nodules (Rhyan et al. 1999; Bondurant 2005).

In the female, 2 weeks after infection, *T. foetus* may have colonized the different parts of the genital tract (Parsonson et al. 1976). The preferred location is in the cervix and cervicovaginal mucus, but the number of parasites varies throughout the estrus cycle, being higher in the days prior to estrus. The establishment of the parasite in the genital tract of the female does not seem to interfere with the fertilization nor with the early development of the embryo (Bielanski et al. 2004). In heifers experimentally infected with *T. foetus*, conceptus deaths peaked at 50–70 days of gestation (Parsonson et al. 1976). Occasional abortions of fetuses older than 4-month gestational age are reported, but typically losses occur 2 months earlier (Bondurant 2005). The infection in the female is usually self-limiting, disappearing between 2 and 4 months after the loss of the conceptus. The immunity that develops is not permanent and usually lasts for about 6 months; after 6 months, the female is again susceptible to infection. Carrier cows—these are cows that remain infected for at least 10 months—seem to fail to develop a protective immune reaction against *T. foetus*. Notable lesions in the maternal endometrium and fetal envelopes have been described only at the time of fetal loss (Parsonson et al. 1976).

The mechanisms of pathogenic actions that underlie the loss of the embryo or fetus are not known with accuracy and may include (1) the direct mechanical action of the parasite, (2) the adverse effects of enzymes secreted by the parasite, and (3) the alteration of the intrauterine environment mainly by antiparasitic inflammatory reactions of an infected dam (reviewed by Bondurant (2005) and Campero and Cobo (2006)). The increase in the number of microorganisms in the female genital tract occurs slowly and probably does not produce any relevant damage until this number exceeds a certain threshold. This fact would explain the long period of time between infection and loss of the conceptus.

14.1.3.2 *T. foetus* Infection in Cats

Few studies have examined the interaction of feline *T. foetus* with intestinal epithelium (recently reviewed by Yao and Köster (2015) and Tolbert and Gookin (2016)). Recent studies examining *T. foetus* infection in a co-culture model with monolayers of porcine intestinal epithelial cells suggest that adhesion to the intestinal epithelium occurs by means of specific receptor-ligand interactions (Tolbert et al. 2013). Pathogenesis of *T. foetus* on the intestinal epithelial cells has been suggested to be both contact-dependent and contact-independent. In the former, a cytopathic effect is mainly exerted via apoptosis induced by cell-associated proteases, whereas extracellular proteases are the major players in contact-independent cytotoxicity. Extracellular proteases may also play a role in evading complement killing.

14.1.3.3 *T. gallinae* and *T. gallinarum* Infections in Birds

The severity of the disease depends on the susceptibility of the infected birds together with the pathogenic potential of the incriminated strain and the stage of infection (Cooper and Petty 1988; Cole and Friend 1999). The severity of pathologic lesions of *T. gallinae* in the upper digestive tract varies from a mild inflammation of the mucosa to caseous areas that block the esophageal lumen (Stabler 1954). Some virulent strains are able to create diphtheritic membranes—associated with fibrinous lesions in internal organs such as the liver, lungs, and peritoneum—resulting in high mortality (Narcisi et al. 1991). Strains of moderate virulence are often associated with caseous abscesses in the upper digestive tract and oropharyngeal region, whereas no appreciable lesions are produced by avirulent strains (Cole and Friend 1999). In vitro, *T. gallinae* proliferation has been associated with a disintegration of the cell monolayer, and genetically different *T. gallinae* isolates caused diverse magnitudes of cytopathic effects on different cell lines (Amin et al. 2012a). However, little is known concerning the mechanism by which *T. gallinae* causes pathological changes in its hosts. Proteolytic proteins secreted by the parasite have been identified as contributing to the detachment of a cell monolayer (Amin et al. 2012b).

Various studies investigated the pathogenicity of *T. gallinarum* either in naturally infected chickens and turkeys or via experimental infection, with contradicting outcomes as reviewed by Amin et al. (2014). Recent studies have shown that in vitro, *T. gallinarum* has no destructive effect on cells and, in vivo, did neither produce clinical signs nor macroscopic or microscopic lesions in turkeys and specified pathogen-free chickens (Amin et al. 2011).

14.2 Clinical Effects and Diagnosis

14.2.1 Clinical Effects

14.2.1.1 Cattle

The clinical effects produced by the disease occur only in female cattle, causing early abortion and temporary infertility (reviewed by BonDurant (1997, 2005, 2007), Yule et al. (1989a), Rae and Crews (2006)). In males, *T. foetus* infection is asymptomatic and affects neither semen quality nor sexual behavior, but bulls can shed the organism indefinitely (Parsonson et al. 1974; Rhyan et al. 1999).

The parasite multiplication causes inflammation of the endometrium, cervical, and vaginal mucous membranes in cows or heifers following the infection at breeding (Parsonson et al. 1976; Rhyan et al. 1988; Anderson et al. 1996). Consequently, signs of mild vaginitis, cervicitis, or endometritis, such as mucopurulent vaginal discharge, may be observed, although generally there are no overt signs. Conception apparently proceeds normally, but almost all conceptuses are lost at some time early in gestation with early fetal death and resorption but also abortion—with a peak loss at 70–90 days (Parsonson et al. 1976; Bielanski et al. 2004). Infection can result in fetal maceration and pyometra. The consequence is infertility (Parsonson et al. 1976; BonDurant 1985; Ball et al. 1987; Anderson et al. 1996). Abortions of fetuses typically occur around 2 months of gestational age. Abortions of fetuses older than 4 months of gestational age due to trichomonosis have been occasionally reported. If the affected cow undergoes early fetal loss, it may cycle regularly without showing any signs but a prolonged interestrous interval (BonDurant 1985). Pyometra occurs in less than 5% of infected cows and is followed—as the *corpus luteum* of pregnancy is maintained—by a large purulent response (Rhyan et al. 1988); pyometra is probably a result of bacterial contamination that occurs at the time of fetal loss, when the cervix is likely to relax sufficiently to admit contamination from outside the environment (Rhyan et al. 1995a). Cows that are infected with *T. foetus* typically clear the infection within a few months, i.e., after three cycles (Parsonson et al. 1976). Immunity, however, is not permanent, and the cow will be subject to reinfection and embryonic death in subsequent breeding periods, and, as mentioned earlier, some infected cows may carry infections into the next breeding season (Skirrow 1987; Mancebo et al. 1995).

In an infected herd, bovine tritrichomonosis is associated with lowered fertility. The usual signs in the herd include return to estrus 1–3 months after breeding. At pregnancy exam time, a number of early pregnancies and open cows are observed. The period of infertility may last for another 2–6 months as a result of the infection. Other clinical features of the disease in the herd include many services per conception, poor pregnancy rates, long calving intervals, and calf crop reduction. In addition, the calving season is spread out causing batches of calves of different ages with a wide variation in weaning weights (Clark et al. 1983a; McCool et al. 1988; Rae 1989; Collantes-Fernandez et al. 2014).

14.2.1.2 Cats

T. foetus is recognized as an important cause of diarrhea in domestic cats. Typical clinical signs in natural infections are chronic or intermittent large bowel diarrhea, which can vary from subclinical to intractable (reviewed by Gookin et al. (1999), Gookin et al. (2001), Foster et al. (2004), Manning (2010), Yao and Köster (2015)). The feces are described as yellow-green in color, gassy, and malodorous with typical signs of colitis including fresh blood, mucus, fecal incontinence, tenesmus, and flatulence. The consistency of the feces can vary from liquid to semi-formed or cow pat (Stockdale et al. 2009). Severe cases may be accompanied by marked inflammation of the anal region, fecal incontinence, and rectal prolapse (Gookin et al. 1999; Foster et al. 2004; Tolbert and Gookin 2009; Bell et al. 2010). In addition, some infected cats have been reported showing systemic signs including anorexia, depression, vomiting, and weight loss (Stockdale et al. 2009). Mortality is extremely rare and only reported in kittens and is presumably caused by endotoxic shock because of deep lesions in the colonic mucosa (Holliday et al. 2009). The majority of infected cats maintain good body condition and appetite without signs of systemic illness (Gookin et al. 1999, 2001; Tolbert and Gookin 2009). The long-term prognosis for *T. foetus*-infected cats is usually good and most will eventually overcome the infection. Remission could take between 4 months and 3 years, with irregular episodes of diarrhea of variable length (Gookin et al. 1999, 2004). No abnormalities are routinely noted on hematology and serum biochemistry profile of some cats, though they remain infected and continue shedding the organism despite clinical improvement, i.e. these cats represent asymptomatic carriers. (Manning 2010). Some positive cats were also infected with other pathogens, like *Cryptosporidium* spp., *Giardia* spp., coccidian, or feline immunodeficiency virus (Gookin et al. 1999; Stockdale et al. 2009); concurrent infections may contribute and increase susceptibility and vulnerability to intestinal disease in infected cats.

14.2.1.3 Other Animals

In birds the two trichomonad species *T. gallinarum* and *T. gallinae* are commonly found (reviewed by Amin et al. (2014)). *T. gallinarum* parasitizes the large intestine of gallinaceous and anseriform birds. *T. gallinarum* induces usually a latent infection in the absence of clinical signs and lesions, and it is not clear whether *T. gallinarum* should be regarded a primary pathogen (Amin et al. 2011; Friedhoff et al. 1991). However, the presence of *T. gallinarum* may aggravate primary diseases—e.g., caused by *Histomonas meleagridis*—and coinfections have been observed (Grabensteiner and Hess 2006). *T. gallinae* is of veterinary and economic importance, as it causes avian trichomonosis, a disease with important medical and commercial implications, which is known as *pigeon canker*, *canker*, *roup*, or *Gelber Knopf* (Amin et al. 2014). *T. gallinae* is located in the upper digestive tract of pigeons, causing lesions (BonDurant and Honigberg 1994). The disease is characterized by greenish fluid and caseous lesions—whitish-yellowish fibrinous material—on the oropharyngeal membranes that can block the lumen of the esophagus impairing drinking and feeding. Clinical signs associated with avian trichomonosis are loss of appetite, vomiting, ruffled feathers, diarrhea, dysphagia, dyspnea, weight loss, increased thirst, inability to stand or to maintain balance, and a pendulous crop

(Narcisi et al. 1991). Death may occur within 3 weeks of infection. Infected birds can also remain asymptomatic due to the infection with avirulent strains of trichomonads or a lower susceptibility as seen in older birds. Avian trichomonosis may also affect domestic fowl; in earlier studies severe outbreaks have been recorded in chickens and turkeys, but we are not aware on recent cases (Hawn 1937).

14.2.2 Diagnosis

14.2.2.1 Diagnostic Techniques

In cattle, the prescribed test for international trade is the identification of *T. foetus* by culture or PCR—World Organization for Animal Health (OIE), OIE Terrestrial Manual. The OIE Terrestrial Manual provides protocols for sampling, sample transportation, transport medium, culture media, culture conditions, and how to read out the culture test. In addition, the OIE Terrestrial Manual also provides recommendations for PCR analyses, which can be applied in combination either with or after culture as an ancillary test or—more often—direct as the primary test to examine bovine samples—i.e., preputial material, uterine or vaginal secretions, or abomasal content of aborted fetuses. Protocols to diagnose bovine tritrichomonosis have been described in detail previously (Sager et al. 2007). To diagnose *T. foetus* infection in cattle, PCR tests may have a higher or at least the same sensitivity as culture tests but have several advantages, because parasites in the sample do not need to be viable and PCR results are rapidly available in contrast to results of culture tests. On the other hand, culture tests are superior to PCR because of their relative easiness (Yao 2013).

In cats, both cultivation and PCR tests are regarded as optimal methods for a sensitive and specific detection of *T. foetus* in fecal samples, although currently PCR is regarded as the gold standard assay for diagnosis of feline *T. foetus* infection since detection is independent from parasite viability (Gookin et al. 2002, 2004; Manning 2010; Yao and Köster 2015). Under optimized conditions, PCR is the method of choice when samples have to be shipped—e.g., from practitioner to a veterinary laboratory. Similar to *T. foetus* in cattle, the success of cultivation tests is largely dependent on the viability of *T. foetus* in the sample (Hale et al. 2009).

In birds infected by *T. gallinae*, an immediate diagnosis by direct microscopy of material collected via swabbing the oral cavity during clinical examination or necropsy is possible. Also, *T. gallinarum* can be observed in fecal material collected from birds—e.g., by swabbing cloacae. However, although the direct detection by light microscopy is fast and inexpensive, it is regarded as insensitive, and low numbers of parasites may not be detected (Amin et al. 2014). Also in birds the use of cultivation for the detection of trichomonads is clearly superior in sensitivity as compared to direct microscopy (Cooper and Petty 1988; Bunbury et al. 2005).

14.2.2.2 Direct Microscopic Examination

In fresh samples it is possible to diagnose the infection with trichomonads by a direct light microscopical examination. Optimal is a 200 to 400-fold magnification. It is advantageous to pre-warm slides, to retain motility of trichomonads. A drop of

physiological saline is added to the slide, mixed with a nearly equal volume of material collected, and mounted with a coverslip.

It is possible to apply conventional light microscopy—either using a conventional up-light or an inverted microscope—, phase-contrast microscopy, or dark-field microscopy. In phase-contrast microscopy, it is easier to see flagella. In dark-field microscopy, trichomonads appear as small rolling luminescent footballs. In conventional light microscopy, trichomonads are identified by their characteristic movement, which is described as rolling and jerky. They are *flashing*, due to their rolling movements. The presence of multiple anterior flagella and the characteristic *refractile* undulating membrane can be observed. However, one disadvantage of direct microscopy is that at the resolution of a conventional laboratory microscope, the exact number of anterior flagella cannot be determined.

The specificity of direct microscopy is very limited. The identification of the trichomonad species observed by this method is not possible, and confirmatory PCR analyses are necessary. For inexperienced examiners it might be difficult to differentiate trichomonads from other intestinal parasites—e.g., *Giardia* spp. in cat feces (Yao and Köster 2015). Also the intestinal commensal trichomonad *Pentatrichomonas hominis* might be misinterpreted as *T. foetus*.

Another disadvantage of direct microscopy is its low sensitivity. A sample is only positive, if it contains a sufficient number of parasites per milliliter specimen. For example, in cats, the fecal examination by direct microscopy is reported to have a diagnostic sensitivity of only about 14%, and also in bovine tritrichomonosis, direct examination is estimated to be 25% less sensitive than culture diagnosis (Gookin et al. 2004; Sager et al. 2007).

The advantage of direct microscopy as diagnostic tool is its speed and the low cost of examination. This is the reason why direct microscopy is often used by practitioners for the examination of *T. foetus* in cats and *T. gallinae* infection in birds (Forrester and Foster 2008; Amin et al. 2014).

Staining of trichomonads is possible using a number of stains, including Giemsa, silver, iron hematoxylin, malachite green, methylene blue, Papanicolaou, and acridine orange (Amin et al. 2011). A fast and inexpensive staining protocol—Giemsa or Diff-Quick and iodine—has been reported (Lun and Gajadhar 1999). Single parasites might be easier to inspect; however, the chance to find small numbers of parasites in a sample might decrease because parasites can no longer be identified by their characteristic movement or *flashing* essential for parasite identification in low concentrated samples. In tissues, the use of hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) stains was proven to be advantageous for identification of the flagellates, especially in organs that contained only a few protozoal cells (Amin et al. 2011, 2014). Immunohistochemical techniques and in situ hybridization have been successfully applied to demonstrate trichomonads in histological sections of cat or bird tissues, respectively (Rhyan et al. 1995b; Yaeger and Gookin 2005; Liebhart et al. 2006; Mostegl et al. 2012). Immunohistochemical detection using monoclonal antibodies against *T. foetus* has been shown to be a valuable tool (Hodgson et al. 1990). A protocol for immunohistochemical detection using the monoclonal antibody Mab 34.7C4.4 is provided in the OIE Terrestrial Manual

(www.oie.int/international-standard-setting/terrestrial-manual). Staining is also applied to confirm positive cultures by morphological criteria.

14.2.2.3 Culture

In vitro culture can be performed by incubating the samples at 25–37 °C in a growth medium. If parasites are present, their numbers will multiply in the culture over time, increasing the likelihood of their detection. A large number of culture systems for trichomonads and especially *T. foetus* have been developed and published. Presumably the first culture system for an axenic cultivation of *T. foetus* isolated from an aborted fetus—i.e., cultivation without bacteria or other living organisms—was reported by a German microbiologist (Witte 1933). From that time on, numerous reports of further cultivation protocols have been published.

Until now diagnostic culturing is of outmost importance for sensitive diagnosis of bovine tritrichomonosis. Also in the diagnosis of tritrichomonosis of cats, cultivation has been widely used for epidemiological studies or diagnostic purposes (Tables 14.2 and 14.3).

In bovine tritrichomonosis cultivation became an important diagnostic tool, because parasite numbers in bovine samples—e.g., preputial smegma or cervico-vaginal mucus—are usually too low to be detected by direct microscopy and a multiplication of parasites after a few days of cultivation increases the chance to find infected bulls.

The number of organisms in preputial secretions has been estimated and ranges from less than 200/mL up to more than 80,000/mL (Skirrow and BonDurant 1988). In bovine tritrichomonosis, diagnostic sensitivity of a single culture test on infected bulls has been estimated to range between 70 and 100% (Skirrow et al. 1985; Schönmann et al. 1994; Parker et al. 1999, 2003a, b). In a large field study, including 2832 mature bulls from 124 beef herds in Argentina, Bayesian estimation revealed a diagnostic sensitivity and specificity of 72.0% (59–87%) and 95.4% (94–96%), respectively (Perez et al. 2006). A repeated testing of bulls—e.g., three times with intervals of several days—has been shown to increase the diagnostic sensitivity of the cell culture test close to 100%. Of 29 samples collected from 5 experimentally infected bulls with resting periods of 2–4 days between samplings, 24 (83%) were determined as positive (Mukhufhi et al. 2003). In another study, consecutive testing over a period of more than 7 months resulted in the determination of an infection rate of 100% in 15 bulls (Clark et al. 1971). For bulls from herds in which *T. foetus* is endemic, two to four tests per bull may be required to ensure that the bull is not infected (Parker et al. 1999). A sexual rest of bulls for a minimum of about 1–2 weeks prior to sampling increases sensitivity (Yule et al. 1989a).

Also for the analysis of females, i.e., after sampling of cervico-vaginal mucus, sensitivity of cultivation is superior to direct microscopic examination (Simmons and Laws 1957; Skirrow and BonDurant 1988). In female cattle diagnostic sensitivity of culture tests has been reported in the range of 56 and 95% (Kimsey et al. 1980; Goodger and Skirrow 1986; Skirrow and BonDurant 1988; Parsonson et al. 1976). The infection in females is usually cleared within 3 months, and it is often difficult to isolate organisms from female cattle in the late stage of their infection.

In cats, the culture method is reported to have a detection limit of about 2×10^2 trophozoites and a diagnostic sensitivity from 26.4 to 58.8% (Hale et al. 2009; Gookin et al. 2004).

To achieve optimal test sensitivity, it is essential to retain as long as possible viability of trichomonads after sampling. The number of viable organisms decreases progressively after sampling (Todorovic and McNutt 1967; Tedesco et al. 1979; Reece et al. 1983; Skirrow et al. 1985; Kittel et al. 1998; Bryan et al. 1999; Parker et al. 1999). Immediate cultivation is ideal but rarely possible. A 1-day delay is estimated to cause a loss of diagnostic sensitivity of 10% (Sager et al. 2007). Sampling the parasite into transportation media providing nutrients has been shown to be essential for the survival of trichomonads, especially if time between sampling and starting cultivation is exceeding 2 days (Kimsey et al. 1980; Hale et al. 2009). An earlier study showed that physiological saline with 5% fetal serum or lactate Ringer's solution was effective (Kimsey et al. 1980). A thyoglycolate transport medium was also shown to be suitable; however, sensitivity of the subsequent cell culture test was slightly lower than after transport using InPouch TF medium (BioMed Diagnostics, White City, OR, USA). Today the medium used for later cultivation is often also used as transportation medium—i.e., InPouch TF or Diamond's medium (Bryan et al. 1999).

An alternative is the direct sampling into a commercially available transport and culture kit—InPouch TF—containing a selective medium, a medium optimized for *T. foetus,* and a medium repressing the growth of the contaminating bacterial flora. This commercial transport and culture kit is recommended not only for sampling in cattle but also for sampling in cats or birds (Thomas et al. 1990; BonDurant 1997; Gookin et al. 2004; Hale et al. 2009; Yao and Köster 2015).

According to the OIE Terrestrial Manual (www.oie.int/international-standard-setting/terrestrial-manual), bovine samples—after being added to transport media—should be protected from exposure to daylight and extremes of temperature, which should remain above 5 °C and below 38 °C (Bryan et al. 1999). For cat fecal samples, a storage for 1 to 24 h at room temperature (23–25 °C) was superior to a 4 °C storage for the same period of time as shown in experiments performed with fecal samples spiked with different *T. foetus* concentrations (2×10^2–2×10^4 *T. foetus* per gram of feces) (Hale et al. 2009).

Several culture media have been found suitable for the cultivation of trichomonads. Overviews on media have been provided in the OIE Terrestrial Manual, www.oie.int/international-standard-setting/terrestrial-manual/, and in several reviews (Skirrow and BonDurant 1988; Sager et al. 2007).

Currently, the most widely used system is InPouch TF, a commercial transport and cultivation kit (Yao 2013). As noncommercial medium the so-called Diamond's medium is widely used, also in epidemiological studies (Tables 14.2 and 14.3). Modified Diamond's medium is a trypticase-yeast extract-maltose medium which in most studies was used modified by the addition of heat-inactivated serum—e.g., of 5% heat-inactivated horse or lamb serum (Diamond 1957; Skirrow and BonDurant 1988; Sager et al. 2007). Both the use of modified Diamond's medium and the

InPouch TF kit are recommended for diagnosis of bovine tritrichomonosis by the OIE Terrestrial Manual (www.oie.int/international-standard-setting/terrestrial-manual, accessed 22. Febr. 2017).

The InPouch TF kit seems superior to Diamond's medium to detect *T. foetus* infection in bulls (Schönmann et al. 1994; Appell et al. 1993; Mendoza-Ibarra et al. 2012; Yao 2013). As regards the cultivating of cat samples, in one study, the InPouch TF kit was found to be superior to modified Diamond's medium (Gookin et al. 2004); however, in a recent study, comparison of both systems revealed a higher sensitivity when modified Diamond's medium was used—ATCC medium 719 (Hale et al. 2009). A retrospective analysis of data revealed no statistical significant differences between cultivation with modified Diamond's medium and InPouch TF.

A modified Plastridge medium containing antibiotics and antifungal agents as well as heat-inactivated bovine serum was recommended for initial cultivation of trichomonads and can be applied combined with modified Diamond's medium for subsequent procedures—e.g., sub-cultivation (Reece et al. 1983; Skirrow and BonDurant 1988; Sager et al. 2007). In studies conducted in Argentina, a commercially available modified Plastridge medium has been applied (Mardones et al. 2008).

The preparation of modified Diamond's medium and test vials, as well as sample processing and reading the results of the culture test, is described in the OIE Terrestrial Manual for bovine samples; many of the recommendations also apply for processing cat and avian samples (www.oie.int/international-standard-setting/terrestrial-manual, accessed 22. Febr. 2017).

Samples collected via preputial scraping—e.g., vigorously by brush, insemination pipette, or aspiration, usually about 0.5–1 mL—can be inoculated directly on top of the medium of a test tube, into the transportation medium, or into the upper chamber of the InPouch TF kit. In contrast, samples collected by preputial washing need to be centrifuged and the supernatant discarded in order to reduce volume. Reading the cell culture tests is performed by microscopic detection of the trichomonads. To increase specificity of the culture test, it is recommended to confirm observed parasites by PCR (Campero et al. 2003).

For cats, voided feces sampled directly from the litter box, rectal swabs obtained from rectal mucous membranes, or feces collected by manual extraction with the aid of fecal loops or by a colon flush technique can be the starting point for trichomonads cultivation (Yao and Köster 2015; Manning 2010; Tolbert and Gookin 2009).

In case of *T. gallinae*-infected birds, a cotton-tipped applicator moistened with sterile saline is used to swab the oral cavity, and swabs are added to InPouch TF culture devices or other commercial or noncommercial culture media (Forrester and Foster 2008; Rogers et al. 2016; Girard et al. 2014; Krone et al. 2005)).

Microscopic detection of culture-growing organisms can be done by light microscopy, on a wet mount slide prepared directly from the culture or through the plastic wall of the InPouch TF kit using a plastic clip provided by the supplier. The motile organisms may be seen under a standard microscope using a 200-fold or higher magnification. An inverted microscope may be useful for examining culture flasks containing culture medium. Culture media should be inspected by microscopic examination at

regular daily intervals—from day 1 to day 7 after inoculation (Bryan et al. 1999; Lun et al. 2000). During the first 4–72 h of culturing, there might be an initial increase of parasite numbers, subsequently followed by a decrease. Organisms may be identified on the basis of characteristic morphological features (OIE Terrestrial Manual, www.oie.int/international-standard-setting/terrestrial-manual, accessed 22. Febr. 2017).

It has been shown for cattle and cats that without test confirmation—e.g., by using specific PCR or sometimes by using staining or electron microscopy—the diagnostic specificity of the culture method remains well under 100%. Therefore, a subsequent PCR analysis of culture-positive samples has been recommended to avoid false-positive findings (Parker et al. 2001; Campero et al. 2003; Ceplecha et al. 2013). Intestinal trichomonads were observed in virgin bull samples submitted for confirmation of InPouch TF culture diagnosis or culture diagnosis using Sutherland medium (BonDurant et al. 1999; Michi et al. 2016). The medium of InPouch TF-Feline is thought to be highly specific to *T. foetus*, and the morphologically similar flagellates *P. hominis* and *Giardia* spp. should not survive longer in this medium than 24 h (Gookin et al. 2003). However, the InPouch TF-Feline medium seems to be not entirely selective as *P. hominis* could be successfully cultivated and identified after 3 days following inoculation of InPouch TF-Feline medium using cat feces (Ceplecha et al. 2013).

14.2.2.4 DNA Detection

DNA detection has become one of the most important methods for the diagnosis of infections with trichomonads in cattle and cats. The major advantage of DNA detection by PCR is that it is independent of parasite viability and contaminating microbes that may inhibit trichomonad cultivation. Correspondingly, sensitivity of PCR is often reported to be higher than cultivation and direct microscopical examination (recently reviewed by Yao (2013)). However, PCR analysis has also a number of disadvantages since due to its sensitivity it is prone to carry-over and cross-contamination. In addition, samples may contain inhibitory components that may reduce the sensitivity of PCR or even disable amplification. Each lab should validate the entire diagnostic process—including DNA extraction and PCR amplification—prior to carry out PCR detection as laboratory-specific conditions, equipment, or consumables may have an impact on the outcome of the diagnostic process (Hoorfar et al. 2004; Conraths and Schares 2006).

Preputial material for DNA extraction is generally collected by sheath scraping combined with aspiration or by sheath washing. Material obtained by scraping may contain blood or fecal contaminations from outside the preputium, and material collected by preputial washing may contain urine. Blood, fecal components, and urine may act as PCR inhibitors, and, therefore, it is necessary to minimize or avoid such contaminations. Analytical sensitivities of PCR protocols are high and usually sufficient to detect the DNA of a single parasite (Table 14.1). In field samples, however, analytical sensitivity might be much lower and has been reported to be around 100 organisms per sample (Mukhufhi et al. 2003). In an assessment of diagnostic sensitivity carried out with spiked cat feces, ten organisms per 200 mg of feces were

Table 14.1 Veterinary relevant diagnostic PCRs to detect trichomonads

Target	Type of PCR	Name of primer	Primer, 5′–3′	Name of probe (type of probe)	Probe (type of probe)	Reported specificity	Reported sensitivity	Remarks	Reference
ITS1/5.8S rDNA/ ITS2	End-point	TFR1, TFR2	TGC TTC AGT TCA GCG GGT CTT CC, CGG TAG GTG AAC CTG CCG TTG G	NA	NA	Amplifies *T. foetus*, *T. suis*, *T. mobilensis*, *T. vaginalis*, *T. gallinae, T. tenax*, *P. hominis* (Felleisen et al. 1998) . No amplification of bacterial DNA orpurified bovine genomic DNA	One or a few protozoa	Also referred to as pan-trichomonad PCR	Felleisen (1997)
ITS1/5.8S rDNA/ ITS2	End-point	TFR3, TFR4	CGG GTC TTC CTA TAT GAG ACA GAA CC, CCT GCC GTT GGA TCA GTT TCG TTA A	NA	NA	Amplifies *T. foetus*, *T. suis*, *T. mobilensis*	One or a few protozoa	Often referred to as *T. foetus*-specific PCR	Felleisen et al. (1998)
18S rDNA, ITS1, 5.8S rDNA	End-point	TF211A, TF211B	CCT GCC GTT GGA TCA GTT TCG TTA, GCG CAA TGT GCA TTC AAA GAT TCG	NA	NA	Does not amplify *Mycoplasma bovigenitalium*, *Ureaplasma diversum*, or bovine genomic DNA	1 pg *T. foetus* DNA	Reported to produce few unspecific DNA bands	Nickel et al. (2002)

(continued)

Table 14.1 (continued)

Target	Type of PCR	Name of primer	Primer, 5′–3′′	Name of probe (type of probe)	Probe (type of probe)	Reported specificity	Reported sensitivity	Remarks	Reference
ITS1-5.8S rDNA-ITS2	End-point	Tricho-F/ Tricho-R	CGG TAG GTG AAC CTG CCG TT (truncated TRF2, (Felleisen 1997)), TGC TTC AGT TCA GCG GGT CT (truncated TRF1 (Felleisen 1997))	NA	NA	Amplifies *T. foetus*, *T. suis*, *T. mobilensis* based on in silico analyses; amplified *Pentatrichomonas hominis*	NA	Used in human samples and in a study on cats (Profizi et al. 2013)	Jongwutiwes et al. (2000) and Duboucher et al. (2006)
18S rDNA, ITS1 and 5.8S rDNA	End-point	Forward, reverse	GTA GGT GAA CCT GCC GTT G (5'FAM labeled), ATG CAA CGT TCT TCA TCG TG	NA	NA	Amplifies *T. foetus* but also trichomonad DNAfrom a variety of genera; *T. foetus* (157 bp), *Tetratrichomonas* spp. (170–175 bp), *Pentatrichomonas hominis* (142 bp)	Accurate typing is possible from both the 1.0 and 0.1 pgtemplates	Using diagnostic size variants from within the internal transcribed spacer 1 (ITS1) region. Incorporation of a fluorescently labeled primer enables high sensitivity and rapid assessment of results for species identification	Grahn et al. (2005)

18S rDNA, ITS1 and 5.8S rDNA	End-point	Forward, reverse 5.8S primer	Forward primer (Grahn et al. 2005); TTC AGT TCA GCG GGT CTT C	NA	NA	Amplified *T. foetus* (367 bp), *Tetratrichomonas* sp. (379 bp), *Pentatrichomonas* sp. (333 bp), *T. gallinae* (364 bp), and *T. vaginalis* (363 bp)	0.1 pg	Analysis in a 2% agarose gel and by using fluorescent-labeled primers and 6% polyacrylamide gels; disadvantage: too much template makes typing difficult or impossible; advantage: low costs	Frey et al. (2009)
ITS1/5.8S rDNA/ ITS2	End-point nested	TFR3, TFR4 (external); TFITS-F, TFITS-R (internal)	TFR3, TFR4, primer sequences published (Felleisen et al. 1998); CTG CCG TTG GAT CAG TTT CG, GCA ATG TGC ATT CAA AGA TCG	NA	NA	*T. foetus*-specific	Sensitivity in PBS: 1 organism, 70% ; 10 organisms, 90%; 100 organisms, 100%; sensitivity in 200 mg of feces: 10 organisms, 90%; 100 organisms, 100%	Single-tube nested PCR	Gookin et al. (2002)
ITS1/5.8S rDNA/ ITS2	Real-time	TFR3, TFR 4	TFR3, TFR4, primer sequences published (Felleisen et al. 1998)	NA	NA	*T. foetus*-specific		SYBR® qPCR	Mueller et al. (2015)

(continued)

Table 14.1 (continued)

Target	Type of PCR	Name of primer	Primer, 5′–3′′	Name of probe (type of probe)	Probe (type of probe)	Reported specificity	Reported sensitivity	Remarks	Reference
5.8S rDNA	Real-time	T.foeForward (TFF2), T.foeReverse (TFR2)	GCG GCT GGA TTA GCT TTC TTT, GGC GCG CAA TGT GCA T	T.foeProbe (5'FAM/3'MGB-NFQ)	ACA AGT TCG ATC TTT G	Amplifies *T. foetus*, *T. suis*, *T. mobilensis*	3 fg DNA, 0.1–1 cells per assay	5' Taq nuclease assay using a 3' minor groove binder-DNA probe; no need for post-amplification processing	McMillen and Lew (2006)
SSU rDNA	End-point nested	External: 16Sl, 16Sr; nternal: TN3, TN4	External published by Cepicka et al. (2005): TAC TTG GTT GAT CCT GCC, TCACCTACCGTTACCTTG; internal: ATA GGA CTG CAA AGC CGA GA, TGA TTT CAC CGA GTC ATC CA	NA	NA	Amplifies *Trichomonas* sp.	NA	NA	Robinson et al. (2010)
SSU rDNA	End-point	Tgf, Tgr	GCA ATT GTT TCT CCA GAA GTG, GAT GGC TCT CTT TGA GCT TG	NA	NA	Amplifies *T. gallinarum*	One protozoon per assay	Cross-reactions with *T. gallinae*. No cross-reactions were also observed with samples from other protozoa (*Toxoplasma gondii*, *Eimeria tenella*, *Cryptosporidium* spp., *E. invadens*, and *E. ranarum*)	Grabensteiner and Hess (2006)

Not reported	End-point + southernblot by probe	TF1, TF2	CAT TAT CCC AAA TGG TAT AAC, GTC ATT AAG TAC ATA AAT TC	Probe for Southern blot	CAT CAT TAA TGC CTT TTG ATG GAT CAG GCA ACC ATT TAT A	Amplifies *T. foetus*	Ten or occasionally fewer protozoa	Southern blot necessary to identify specific band. A 400 bp product from bovine genomic DNA is amplified. Multiple amplification products from DNA from a related organism, *T. vaginalis*; Southern blot is negative for *T. vaginalis*	Ho et al. (1994)

NA not applicable

detected in 90% of nested PCR tests, and 100 organisms per 200 mg of feces were detected in 100% of nested PCR tests (Gookin et al. 2002).

It has been shown that also in PCR diagnosis the likelihood to detect *T. foetus* decreases with time between sampling and analysis. In one study it was reported that diagnostic sensitivity in PCR detection declined from 90% when samples were stored for 6 h to 31% when they were stored for 5 days (Mukhufhi et al. 2003). It has been hypothesized that hydrolases secreted by trichomonads are responsible for this effect (Thomford et al. 1996; Sager et al. 2007). Adding the DNA-stabilizing agent guanidinium thiocyanate—GuSCN in a final concentration of 200 mmol/L—or a commercial lysis buffer for sample collection known to preserve DNA for several months at room temperature to the transport medium did not improve results (Mukhufhi et al. 2003; Mendoza-Ibarra et al. 2012). Fecal samples from cats should be submitted within 24 h after sampling at room temperature or 4 °C.

To prevent amplification of carry-over contaminants, protocols that incorporate dUTP instead of dTTP as a nucleotide and allow to apply the Uracil-DNA Glycosylase (UDG) system have been reported (Longo et al. 1990; Felleisen et al. 1998). Contaminating amplicons carried over from previous PCRs can be removed by this system before a PCR amplification. Possible disadvantages of the UDG system are that it might become necessary to optimize the reaction mixture used for PCR—e.g., the $MgCl_2$ concentration—and a standard PCR buffer may not work (Felleisen et al. 1998).

It is essential to monitor the presence of a potential inhibitor in each individual sample since preputial material, cervico-vaginal mucus, and fecal samples contain PCR-inhibiting components. There are different possibilities available. The TFR3/TFR4 PCR protocol includes an artificial internal control DNA carrying TFR3 and TFR4 sequences—based on pBluescript KS+ DNA—and generates control amplicons, via PCR amplification and composite primers (Table 14.1) (Felleisen et al. 1998; Sager et al. 2007). Internal controls of unrelated DNAs have been integrated into a 5′ nuclease assay—real-time PCR assays with TaqMan probes. In this type of assay it is possible to incorporate an unrelated DNA into the sample prior to DNA purification, which is later amplified in a multiplex assay along with the parasite DNA but detected by a probe carrying a fluorophore different than that of the parasite-specific probe. One commercially available *T. foetus* real-time PCR includes such a control system (VetMAX™-Gold Trich Detection Kit, Life Technologies). This principle of inhibition control is becoming more and more popular, also for in-house assay.

There are many ways, by which DNA from preputial smegma, cervico-vaginal mucus, fecal samples, oropharyngeal swabs, culture material, and others types of samples can be extracted. Often commercial kits but also in-house methods have been applied. Also non-purified but heat-treated samples were used with success (McMillen and Lew 2006). However, the use of unpurified DNA is prone to inhibition and is not generally recommended. It has been shown that inhibiting components could be successfully removed from preputial smegma by 5% Chelex®-100 and 0.05% agar solution (Chen and Li 2001). However, in another study, the use of Chelex®-100 caused significantly lower detection rates (Mendoza-Ibarra et al. 2012).

The majority of published diagnostic PCRs for *T. foetus* are targeting rRNA-coding genes (rDNA) and their flanking regions (Table 14.1). These regions include the 18S rRNA gene, the internal transcribed spacer (ITS)1 region, the 5.8S-rRNA gene, the ITS2 region, and the 28S rRNA gene. One of the first diagnostic PCRs established—the TFR3/TFR4 PCR—is widely used. The TFR3 primer targets the 3' end of the 18S rRNA gene and the TFR4 primer the 5′ end of the 28S rRNA gene (Felleisen et al. 1998). Although this PCR assay is often referred to as being specific for *T. foetus*, also DNA of *T. mobilensis*—an intestinal parasite of squirrel monkey—or *T. suis* is amplified (Table 14.1).

The rRNA gene sequences have been widely used for phylogenetic studies in Parabasalia to which trichomonads belong. Other genes coding for cysteine proteinases—CP1, CP2, and CP4–CP9—and cytosolic malate dehydrogenase 1 (MDH1) have been used to differentiate *T. foetus* isolates from cattle and cat or to characterize new strains of *T. foetus* (Kleina et al. 2004; Cepicka et al. 2005, 2006; Gaspar da Silva et al. 2007; Kolisko et al. 2008; Sun et al. 2012; Slapeta et al. 2012; Casteriano et al. 2016). In *T. gallinae* further genes were used to define lineages (recently reviewed in Amin et al. (2014)).

Because 18S rRNA genes show limited differences between trichomonads, endpoint assays have been applied using primers capable to amplify DNA of several trichomonad species simultaneously (Felleisen 1997). In these PCRs, species diagnosis was achieved in a second step, either by PCR-RFLP, by determination of the precise size of amplification products, or by single-strand conformation polymorphism (SSCP) (Hayes et al. 2003; Huby-Chilton et al. 2009).

The TFR3/TFR4 PCR protocol has been modified by using the TFR3/TFR4 primer pair for external amplification followed by an internal newly designed primer pair in a single-tube nested PCR (Gookin et al. 2002). The TFR3/TFR4 PCR protocol has been also modified into a SYBR®-based real-time PCR assay (Mueller et al. 2015).

A 5' nuclease assay—i.e., a real-time PCR applying a TaqMan probe—based on rRNA gene sequences has been established to detect *T. foetus*, *T. suis*, and *T. mobilensis* (McMillen and Lew 2006). In this assay a 57 bp region of the 5.8S rRNA gene region is amplified (Table 14.1). As mentioned earlier in this section, a commercialized 5'' nuclease assay is available (VetMAX™-Gold Trich Detection Kit) which has been used in epidemiological studies on *T. foetus* of cattle in Southern Africa (Casteriano et al. 2016).

14.2.2.5 Serological Techniques

Serological and other antibody detection tests have been established. However, they are of no importance for the diagnosis of trichomonosis. These tests include:

- **Agglutination and hemolytic tests.** In *T. foetus*-infected cows, antibodies appear in the cervicovaginal mucus about 6 weeks after infection and persist for several months (Pierce 1947). A mucus agglutination test detected 32% (57 of 178) of cows in naturally infected herds, and no cows from clean herds tested positive (Pierce 1949). The mucus agglutination test was shown to be

specific as no cross-reactions with *Campylobacter fetus* or *Brucella abortus* has been observed. However, the reliability of the test was strongly influenced by the type of mucus. The mucus agglutination test was regarded as herd test (Pierce 1949).

- A serum agglutination tests, similar to the mucus agglutination test, did not show results that correlated well with the *T. foetus* infection status of cows (Kerr 1944).
- A hemolytic assay has been established which showed in female cattle a diagnostic specificity of 96% and a diagnostic sensitivity of 94% (BonDurant et al. 1996). However, only 43% of chronically infected bulls tested positive when this test was applied (BonDurant et al. 1996).
- **Indirect ELISAs to detect parasite-specific antibodies.** An indirect ELISA (iELISA) based on the TF1.17 surface antigen of *T. foetus* showed promising results when tested with cervico-vaginal mucus of heifers (Ikeda et al. 1995). TF1.17 surface antigen-specific IgG1, IgA, and IgM antibodies in the smegma of bulls naturally infected with *T. foetus*—as determined and measured by ELISA—were observed concurrently with *T. foetus*-positive smegma cultures (Rhyan et al. 1999); to the best of our knowledge, this approach was not further elaborated. In vaccination studies, IgG1, IgG2, IgA, and IgE responses were monitored in the preputial secretions or in sera of bulls by using a whole *T. foetus* cell antigen preparation for ELISA (Cobo et al. 2009).
- An iELISA coated with whole *T. foetus* parasites and fixed with ethanol was used to determine an isotype-specific antibody response in the reproductive tract secretions and sera of *T. foetus*-infected heifers (Skirrow and BonDurant 1990a). In cervical and vaginal secretions, parasite-specific IgA and IgG1 antibodies predominated 7–12 weeks after infection, while in serum, parasite-specific IgG1 and IgG2 antibodies were detected. Interestingly, elevated antibody levels were observed after reinfection using this iELISA (Skirrow and BonDurant 1990a). A similar iELISA with immobilized whole *T. foetus* parasites was used to monitor the *T. foetus*-antibody response in immunized heifers naturally challenged by being bred with a naturally infected bull (Cobo et al. 2002). The presented serological tests have not been used or validated for routine diagnostic purposes.

An iELISA has been also used under experimental conditions to detect antibodies against *T. gallinarum* and *T. gallinae* in poultry (Amin et al. 2011). For this iELISA, the plates were coated with *T. gallinarum* parasites in carbonate buffer per well.

14.2.2.6 Intradermal Test

Analogous to the tuberculin test, a Tricin test has been developed to identify *T. foetus*-infected cattle or herds (Kerr 1944). The antigen used for an intradermal Tricin test has been prepared by fixation of cultured *T. foetus* parasites using trichloroacetic acid. Skin reactions—i.e., an increase in skin thickness—were read 30–60 min after the application of the antigen. This test was regarded to represent a herd test (Kerr 1944).

14.2.2.7 Antigen Detection

Attempts to develop a sensitive and specific diagnostic antigen test for detecting *T. foetus* antigen in cervico-vaginal mucus have failed (Yule et al. 1989b). However, immunohistochemical techniques have been successfully applied to demonstrate trichomonads in histological sections (Rhyan et al. 1995b; Yaeger and Gookin 2005). For immunohistochemical detection, monoclonal antibodies developed to characterize *T. foetus* antigens revealed to be valuable tools (Hodgson et al. 1990). A protocol for immunohistochemical detection by using the monoclonal antibody Mab 34.7C4.4 is provided in the OIE Terrestrial Manual (www.oie.int/international-standard-setting/terrestrial-manual, accessed, 22. Febr. 2017).

14.2.2.8 Diagnosis in Different Hosts

Diagnosis in Cattle

A tentative diagnosis of trichomonosis as a cause of reproductive failure in a herd is based on the clinical history, signs of early abortion, repeated returns to service, or irregular estrous cycles. Confirmation of infection depends on the demonstration of the organism in placental fluid, stomach contents of the aborted fetus, uterine washings, pyometra discharge, vaginal mucus, or preputial smegma. In infected herds, the most reliable material for diagnosis is preputial scrapings (Kittel et al. 1998; Mukhufhi et al. 2003; Parker et al. 1999; Schönmann et al. 1994).

Bulls are the main reservoir for the parasite. Control programs focus on identifying and culling infected bulls and nonpregnant cows carrying the parasite. Prevention of transmission of the disease through culling practices relies on the ability to identify infected animals accurately.

Advances in cell culture and polymerase chain reaction (PCR) have increased the ability to detect the disease in bulls. However, the collection of an adequate sample is of outmost importance, as this step affects sensitivity, specificity, and repeatability. The low repeatability observed with most sample collection techniques can cause false-negative results. The most efficient method of sampling vaginal and preputial secretions is insertion of an insemination/infusion pipette inside the vaginal fornix or preputial cavity and performing short strokes while concurrently aspirating secretions (Cobo et al. 2007). Vagina or preputial cavity can be washed with PBS to recover more organisms, although this usually dilutes the sample. Alternatively, preputial secretions can be collected by scraping with a plastic or metal brush, with no significant differences in culture sensitivity compared to using a pipette (Tedesco et al. 1979; Parker et al. 1999).

Diagnosis in Bulls

Routine herd diagnosis is optimally performed on bulls and not on females, because bulls remain permanently infected while in most female cattle infection is only transient. In addition, sampling of bulls reduces costs as preputial samples have to be taken only from a smaller number of bulls. Diagnosis in the bull involves collecting, transporting, and culturing the sample in special growth media and tentatively identifying the organism by microscopic examination. Finally a confirmation of detection using PCR has to be carried out to confirm a *T. foetus* infection (Fig. 14.4). If samples

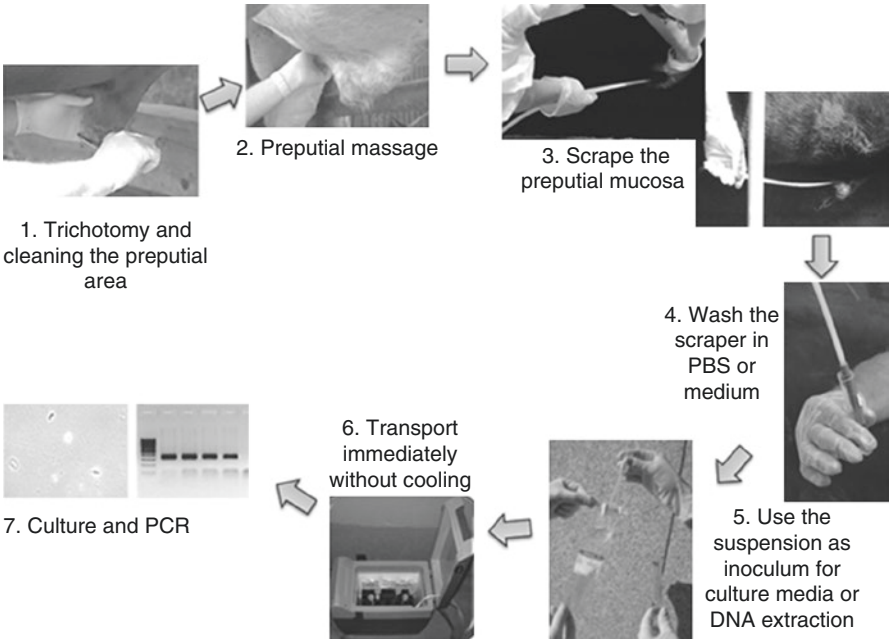

Fig. 14.4 Collection of preputial smegma samples and diagnostic methods for bovine tritrichomonosis

are processed by PCR, the use of pooled direct preputial samples is possible. However, this strategy required repeated sampling to optimize sensitivity (Garcia Guerra et al. 2013). Optimally, all bulls belonging to the same herd should be sampled. In those herds in which periodic sanitary control is carried out without previous history of venereal diseases and with good pregnancy rates, at least two consecutive samplings of all bulls should be performed, with a minimum of 1 or 2 weeks time between them (Skirrow et al. 1985; Kimsey et al. 1980). Consecutive testing is necessary due to the low diagnostic sensitivity of an individual test. If both samplings revealed negative results, the herd can be considered tritrichomonosis free.

Some recommendations have to be taken into account at the time of sampling:

- It is generally recommended to allow a sexual rest of about at least 1–2 weeks before taking a preputial sample to allow a repopulation of microorganisms in the preputial cavity and increase diagnostic sensitivity.
- It is important to have adequate facilities to perform preputial sampling particularly when sampling a large number of bulls to avoid accidents and injuries.
- The bulls should remain in a pen, without access to water approximately 12 h prior to sampling. This will prevent urination during sampling.
- The external preputial area must be cleaned with disposable paper towels without soap or disinfectants, and—if necessary—preputial hairs should be trimmed to prevent contamination.

The presence of *T. foetus* seems to be confined to the preputial cavity, and *T. foetus* is localized in the preputial secretions and does not invade the epithelium of the penis or prepuce (Parsonson et al. 1974). *T. foetus* could not be cultured from the epididymis, ampulla, seminal vesicle, pelvic urethra, or testis. Macroscopic and microscopic examination of the genital tracts of infected bulls did not reveal any lesions populated by *T. foetus* (Parsonson et al. 1974).

A number of techniques for collecting preputial samples from bulls have been described (INTA, 2014, Técnicas de muestreo para el diagnóstico de enfermedades venéreas en bovinos, https://www.youtube.com/watch?v=G-1StrWaHKA, accessed 21. Febr. 2017; Navajo Technical College, 2012, Veterinary Technicians perform Trich testing, https://www.youtube.com/watch?v=lp8fpDVDOCE, accessed 21. Febr. 2017). In all these protocols, it is important to avoid contaminations, as this may introduce intestinal protozoa or PCR-inhibiting contaminants. Samples can be collected from bulls by scraping the preputial and penile mucosa with an artificial insemination pipette or scraper, by preputial lavage, or by washing the artificial vagina after semen collection (Cobo et al. 2007; Tedesco et al. 1979; Parker et al. 1999). The latter technique is not recommended as its sensitivity may be lower (Ostrowsky et al. 1974; Parker et al. 1999).

The collection of the preputial smegma can be carried out by using different devices:

- A *Cassou* insemination pipette (Cassou straws, IMV Technologies, L'Aigle, France) covered by a plastic sheath. Inside the preputial cavity, the pipette is pulled forward through the plastic sheath to expose the tip and moved back and forward in short strokes adjacent to the glans penis, especially near the fornix, while aspirating and massaging the glands penis to release greater amounts of smegma into the pipette. A new plastic sheath is used for each bull (Cobo et al. 2007).
- A sterile, dry, plastic *insemination/*infusion pipette of 43 cm length with a 10–15 mL syringe attached to one end is placed into the preputial fornix. The pipette is scraped vigorously across the preputial epithelium without aspiration, and then negative pressure is applied with the syringe to collect preputial smegma. The negative pressure is released before removing the pipette from the sheath, to avoid aspiration of urine or other contaminants. After removing the pipette from the sheath, the sample is placed immediately into a transport or culture medium. A new syringe and pipette are used for each bull (Rodning et al. 2008).
- A plastic or metal *scraping instrument* of approximately 70 cm in length, having at the anterior end a grooved surface of approximately 10 cm in length by 0.8 cm in diameter, which is used to perform scraping of the preputial mucosa. The scraper is introduced into the preputial mucosa by performing 20–30 movements back and forward. It is then carefully withdrawn from the preputial cavity, avoiding contamination with the external part of the foreskin (Terzolo et al. 1992). The aspiration method for the collection of preputial secretions for cultural examination continues to be used although a specially designed scraping instrument was

reported to possess advantages (Bartlett et al. 1947; Clark et al. 1971; Stuka and Katai 1969).

- A *gauze sponge*, which to our knowledge is rarely used. In this procedure, the penis is extended by electrostimulation with a rectal probe. Once extended, a 16-ply gauze sponge is used to wipe around the glans and down the penile shaft and exposed preputial mucosa two to three times. Recently, it has been demonstrated that sponge sampling of *T. foetus* from bulls is a valid method (Dewell et al. 2016). The method facilitates easier collection once the penis is extended and is potentially safer for the veterinarian and the bull. Additionally, the gauze sponge method might be slightly more sensitive than the pipette method (Dewell et al. 2016).

After collection the material has to be inoculated in corresponding transport or culture media and should arrive at the laboratory within 24–36 h; during transportation, the sample should be protected from exposure to daylight and extremes of temperature.

Diagnosis in Heifers and Cows

Initially, the uterus was regarded as the definitive and persistent site of infection while the vagina was considered to be a relatively unreliable source of *T. foetus* for diagnosis (Bartlett 1947). Subsequently, however, the persistence of *T. foetus* in the vagina and/or cervix for periods up to 95 days post-infection was shown, and it was established that samples collected from vagina and the uterine cervix allow a reliable and accurate diagnosis (Parsonson et al. 1976). More recent studies of naturally infected cows reconfirmed that the cervix belongs to the preferred site of parasite location (Skirrow and BonDurant 1990b). The numbers of parasites present in the cervical-vaginal mucus fluctuate during the estrus cycle, and the largest numbers are seen a few days before estrus.

The time a cow remains infected was significantly longer for cows experiencing their first, mean 20.3 weeks, than for those experiencing either their second, mean 9.8 weeks, or their third period of infection, mean 11 weeks. However, the rate of isolation of *T. foetus* from samples of vaginal mucus collected from cows remained similar—mean 83.5%—irrespective of the period of infection or whether the cows showed normal fertility, infertility, or abortion (Clark et al. 1986).

The efficiency of diagnosis in cows increased with temporal proximity between the initial infection and the time of sampling (Clark et al. 1986). It is important to note that the detection of positive females is of value for the initial detection of the infection in a herd. However, it is not useful for a subsequent disease control because cows usually clear their infection and generally become immune, at least for the actual breeding season (BonDurant 1997; Fitzgerald 1986). Sampling should be performed when females with conception failures are observed or at the time of rectal palpation in nonpregnant females. Culture sensitivity is lower in cows than in bulls. However, it is important to point out that the optimal period for sampling is near the end of the service period (Skirrow and BonDurant 1990b; Terzolo et al. 1992).

Uterine and vaginal secretions can be collected in cows that have aborted, in those that have not been pregnant, or heifers. To perform the extraction of cervico-vaginal mucus, a sterile, dry, plastic 43 cm insemination pipette with a

10 mL syringe attached to one end or a Cassou insemination pipette (44 cm long × 0.64 cm outer diameter × 0.32 cm inner diameter; Cassou straws, IMV Technologies, L'Aigle, France) can be used. Opening the lips of vulva without having to fix the cervix rectally, the pipette is inserted in a dorsal-cranial direction into the vagina. After the pipette has been inserted, slight anteroposterior and circular movements are executed, performing aspiration simultaneously. The vacuum generated is usually sufficient to extract the cervical-vaginal mucus, which will vary in quantity and consistency depending on the moment of the estrous cycle in which it is extracted. In a low percentage of animals, the volume of mucus extracted may be scarce. In this case it is possible to introduce 5 mL of phosphate-buffered saline solution by performing a wash with subsequent extraction.

The use of a "screwhead scraper rod" for collecting of samples from the cervico-vaginal mucosa proved to be a practical method and calls for further comparative evaluation with other standard methods in use (Abbitt and Ball 1978). Apart from other extraction techniques, also the Bartlett glass pipette procedure, which is rarely used because it is complicated and the equipment often inaccessible, has been described (Hammond and Bartlett 1943).

Fetal Diagnosis

When abortion occurs, *T. fetus* can be isolated from placental fluids or cotyledons. However, the high degree of contamination of this material limits its use. Isolations can be made from samples taken from the fetal mouth. Swabbing the mucosa of the tongue and roof of the mouth has been recommended (Case and Keefer 1938). Nevertheless, the place where *T. foetus* is most consistently isolated is the abomasal fluid. The sample can be taken with sterile syringe and needle. Once the abomasum content has been extracted, it can be sent to the laboratory in the same syringe or can be inoculated in transport or cultured medium.

Diagnosis in Cats

The diagnosis of feline trichomonosis has been reviewed comprehensively (Tolbert and Gookin 2009; Manning 2010; Yao and Köster 2015). *T. foetus* infection is suspected in cats with recent—less than 6 months lasting—clinical signs of chronic large bowel diarrhea, in young, purebred cats, from densely housed origin. Routine coprological methods, like flotation-sedimentation or sodium acetate-acetic acid-formalin concentration (SAF), destroy or fix trichomonads, respectively, and fixation causes the loss of their characteristic movement which makes them hard to be recognized.

Currently, the preferred diagnostic methods for feline trichomonosis include visualization of the organism in direct smears or culture or *T. foetus* DNA detection by PCR (Gookin et al. 2001, 2002, 2004; Levy et al. 2003; Foster et al. 2004). Histopathological examinations of colon, cecum, and ileum samples are not routinely used but can be helpful to diagnose a feline tritrichomonosis.

It is important to mention that parasite shedding in feces is erratic throughout the course of infection being occasionally so low that it cannot be detected by

diagnostic techniques. Consequently, it is advisable to resample cats showing clinical signs that have been tested negative or to increase diagnostic sensitivity by using more than one test method—e.g., culture and PCR (Gookin et al. 2002). Cats should not receive any antibiotics within several days prior to or at the time of testing.

Samples consist of fresh voided feces taken directly from the litter box, rectal swabs, and manual collection with the aid of fecal loops or by a colon flush technique (revised in Manning (2010), Yao and Köster (2015), Tolbert and Gookin (2009)). Samples collected with a *fecal loop* or by the *colon flush* technique are preferable. The technique of colon flush is demonstrated in a video clip the North Carolina State University, College of Veterinary Medicine website (http://www.youtube.com/watch?v=JMfZ9M80V8E, accessed 22. Febr. 2017). Freshly voided or diarrheic feces are considered ideal for testing whereas samples obtained from normal or dry stools are believed to be less suitable (Tolbert and Gookin 2009).

A diagnostic approach based on direct fecal smears, which may reveal motile trophozoites, can be employed during examination at pet clinics. Samples are suspended in saline and examined immediately under a cover slip at 200 to 400-fold magnification using a light or, preferably, a phase-contrast microscope (Fig. 14.5). Although direct smears represent a cheap, quick, and readily available technique, the sensitivity of microscopic examination of a direct fecal smear is low. The diagnostic sensitivity of a direct smear using samples from naturally infected cats has been shown to be only 14% in one study, but sensitivity can be increased by analyzing multiple fecal smears (Gookin et al. 2004). Other challenge associated with this diagnostic procedure is the skill of the practitioner in identifying motile trichomonads. *T. foetus* cannot precisely be distinguished by microscopical examination from *P. hominis* and is often misdiagnosed as *Giardia* spp. (Tolbert and Gookin 2009; Manning 2010; Yao and Köster 2015). A video clip demonstrating the classic jerky motility has been provided by the North Carolina State University, College of Veterinary Medicine website (https://www.youtube.com/watch?v=aF06jlbcF8E; accessed 22. Febr. 2017). The presence of *Giardia* spp. can be confirmed by a fecal enzyme-linked immunosorbent assay for *Giardia*-specific antigen. Differentiation

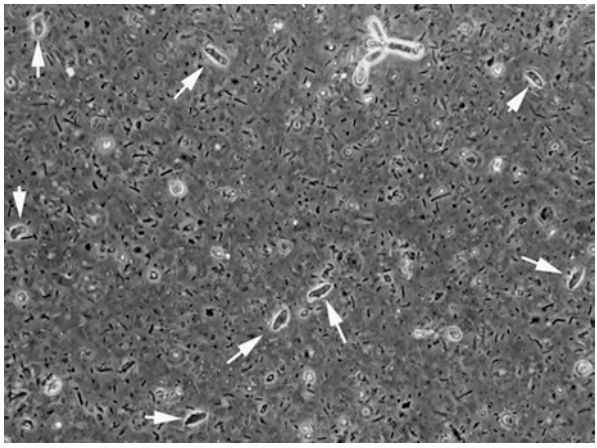

Fig. 14.5 Microscopic detection of *Tritrichomonas foetus* trophozoites (*arrows*) in feline feces (200× magnification)

of *Giardia* spp. and *T. foetus* is crucial to avoid a useless initiation of tritrichomonosis therapy using potentially neurotoxic ronidazole.

In vitro culture of *T. foetus* can be started by incubating the sample feces in a suitable growth medium (Gookin et al. 2001, 2003). Diagnostic kits—for example, the InPouch TF-Feline test kit—can be an attractive option for the clinical practice due to the commercial availability, a shelf life of a year, and the simplicity of use. Sample submission requirement for in vitro culture diagnostic includes the collection of fresh feces—roughly the size of a lima bean—and the use of transport-growth media, InPouch TF or modified Diamond's media. It is recommended to dilute the fecal samples in PBS or physiological saline—to about 1 to 20 v/v—to create a homogenous mixture with a semiliquid consistency to be used as the inoculum for the culture medium, reducing the bacteria burden in the sample (Arranz-Solis et al. 2016). Furthermore, it is crucial for a reliable diagnosis of feline trichomonosis to keep the time delay from sample collection to processing as short as possible, given the environmental fragility of *T. foetus* trophozoites (Gookin et al. 2004). In order to maximize the diagnostic sensitivity, cat feces should be inoculated into the culture within a 6-h period after voiding and to be submitted at room temperature—23 to 25 °C—to a specialized diagnostic laboratory within 24 h (Hale et al. 2009). False-negative findings can be due to refrigeration or delayed processing of feces.

After culture initiation, samples are then incubated at room temperature or at 37 °C; incubation at room temperature made more robust and long-lived cultures (Gookin et al. 2003). Cultures are examined microscopically periodically up to 7 days—normally every 24–48 h—on a wet mount slide prepared directly from the culture or through the plastic wall of the InPouch TF kit. Most of the microscopically positive culture samples are detected 72 h post-incubation. It has to be mentioned that the diagnosis by culture is more difficult for cats than for bovines due to the nature of the feces sample. As feces are directly cultured in enriched media, bacterial and/or fungal contamination is more likely to arise. Bacterial contamination severely impairs the culture of *T. foetus* and is able to reduce the sensitivity of the culture method. Incubation at 37 °C shows a quicker positive result but also more bacterial overgrowth potentially inhibiting the growth of *T. foetus*. If the commercial InPouch TF-Feline test kit is used, an accumulation of gas within the pouches due to an overgrowth of fecal flora is a commonly observed problem. Moreover, *T. foetus* and related parasites are hard to distinguish with light microscopy because of the similar morphology. Culture-positive samples require confirmation by PCR because also other trichomonads like *P. hominis* are able to grow (Ceplecha et al. 2013).

Molecular diagnosis is becoming more widely available than culturing the organism. Under optimized conditions, a direct diagnosis by PCR—i.e., without a prior cell culture test—offers a highly specific and a sensitive diagnostic alternative also able to detect nonviable parasites. Moreover, PCR is the method of choice to confirm positive results by culture. It is important to monitor a potential PCR inhibition in each individual sample. The choice of an appropriate DNA extraction technique greatly influences the reliability and sensitivity of PCR (Stauffer et al. 2008; Hale et al. 2009). Several methods have been successful employed for feline

trichomonosis diagnosis, such as a modified procedure using the commercial QIAamp DNA stool minikit (QIAGEN, Hilden, Germany), Boom's method, or ZR Fecal DNA kit ZR (Zymo Research, Orange, CA, USA), which was able to detect 10 *T. foetus* organisms per 100 mg feces in 100% of PCR reactions (Stauffer et al. 2008). Diagnostic *T. foetus* PCR using primers TFR3/TFR4 and a single-tube nested PCR using primers TFITS-F/TFITS-R in combination with primers TFR3/TFR4 are widely used for feline trichomonosis diagnosis (Table 14.1) (Felleisen et al. 1998). PCR amplification of DNA extracted from feces samples appears to be more sensitive than the InPouch TF culture, and a high level of agreement has been described between the culture and PCR detection (Gookin et al. 2002; Hosein et al. 2013; Arranz-Solis et al. 2016).

Finally, colonic histopathology can be used as a diagnostic tool. Colon, cecum, and ileum samples are collected during necropsy, surgery, or endoscopy. On histologic examination, large numbers of teardrop- to crescent-shaped trichomonads can be found associated with the colonic mucosal surface and less commonly within colonic crypt lumens. Histologic features include mild to moderate lymphoplasmacytic colitis with crypt micro-abscesses, increased mitotic activity, loss of goblet cells, and attenuation of superficial colonic mucosa (Yaeger and Gookin 2005). The probability of diagnosing a *T. foetus* infection based on histopathology increases with the number of submitted samples—a minimum of six colon samples is required to have a larger than or equal to 95% confidence of detecting *T. foetus* in at least one sample (Yaeger and Gookin 2005). Recently, a fluorescence in situ hybridization assay (FISH) and a chromogenic in situ hybridization (CISH) technique have been described to allow the localization and identification of *T. foetus* in formalin-fixed and paraffin-embedded samples, respectively (Gookin et al. 2010b).

Diagnosis in Other Animals

The trichomonad *T. gallinae* of birds is of veterinary importance, while *Tetratrichomonas gallinarum* is a commensal that can become important under certain circumstances—e.g., concurrent infections with other pathogens (recently reviewed in Amin et al. (2014)). Diagnostic techniques described for *T. foetus* can be also applied to the diagnosis of bird trichomonosis, including direct microscopy, cultivation, and PCR detection.

For direct microscopy, sample material can be collected by swabbing the oral cavity, *T. gallinae*, or cloacae, *T. gallinarum*, and is mounted either diluted or undiluted on glass slides. Glass slides need to be examined immediately to observe motile protozoa (Forrester and Foster 2008; Amin et al. 2014). As mentioned, staining of trichomonads is possible and may facilitate detection (Amin et al. 2011). Similar to bovine and feline trichomonosis, the sensitivity of direct microscopy is low, and situations in which the parasite load is low may likely result in false-negative results.

The use of samples to initiate cultures allows the amplification of the number of parasites making this procedure more sensitive than the direct microscopic examination of samples. Correspondingly, in a study of pigeons, only about half of the

samples positive in culture revealed a positive result in wet mount microscopy (Bunbury et al. 2005). In case of *T. gallinae*-infected birds, material collected via swabbing from the oral cavity of the animal is added to a InPouch TF culture device or other commercial or noncommercial culture media (Bunbury et al. 2007; Forrester and Foster 2008; Rogers et al. 2016; Girard et al. 2014; Krone et al. 2005). For cultivation, specimens are usually incubated at 37 °C for several days and examined microscopically every 24 h. Although birds have a higher body temperature, 37 °C seems to be the optimal temperature for the cultivation of *T. gallinarum* (De Carli and Tasca 2002; De Carli et al. 1996; Amin et al. 2010).

For PCR, a number of primer pairs have been reported that target the ITS1-5.8SrDNA-ITS2 genomic region but which are not species-specific and able to detect virtually all trichomonad species. Most commonly used are primers TFR1 and TFR2 (Felleisen 1997). A nested PCR, targeting the 18S rRNA gene, ITS1, and 5.8S rRNA gene, was established but was not species-specific, amplifying also *T. gallinae* (Frey et al. 2009; Grahn et al. 2005). Another nested PCR has been developed for the amplification of the ITS1-5.8S rDNA-ITS2 genomic region of trichomonads used to detect *T. gallinae*-specific DNA from esophageal lesions of finches sampled during an epidemic of finch mortality (Robinson et al. 2010; Cepicka et al. 2005). The analytic specificity of this PCR has not been reported. Amplification primers specifically designed for the identification of *T. gallinarum* yielded also a PCR product of specific DNA of *T. gallinae* of identical size (Grabensteiner and Hess 2006).

An indirect ELISA has only been used under experimental conditions to detect antibodies against *T. gallinarum* and *T. gallinae* in poultry (Amin et al. 2011). Because birds are often latent carriers of trichomonads, there is hope that serological analyses in bird populations might be able to identify carrier birds and may help to better understand persistence and spread of these parasites (Amin et al. 2014).

14.3 Epidemiology

14.3.1 Epidemiology of Tritrichomonosis in Cattle

Bovine tritrichomonosis is a major problem worldwide, affecting a large proportion of herds in North and South America, in parts of Europe, Africa, Asia, and Australia (de Oliveira et al. 2015; Yao 2013; Perez et al. 2006; Mendoza-Ibarra et al. 2012, 2013; Madoroba et al. 2011; Yang et al. 2012; Guven et al. 2013; McCool et al. 1988). Bovine tritrichomonosis is considered endemic in herds managed under extensive conditions and using natural service for breeding (Bondurant 2005). The economic impact of tritrichomonosis infection has to be regarded severe in particular regions of the world. The calf crop in affected beef and dairy herds can be reduced up to 50% in beef operations (Rae 1989). Economic losses are variable and depend on the percentage of bulls infected and the susceptibility of the cows in the herd. Further losses, in addition to calf crop losses, include an extended breeding season due to an increased number of repeat breeders. Due to later calving, the

growing periods for calves might be shortened, and there are batches of calves of different ages with a wide variation in weaning weights. Summarizing these losses, it was estimated that tritrichomonosis in a herd caused a 35% decrease in economic return per cow in an infected herd (Rae 1989). Economic losses in a case of bovine tritrichomonosis in a large Californian dairy herd were calculated at 665 US$ per infected cow (Goodger and Skirrow 1986).

The number of tritrichomonosis reports has drastically reduced in regions or production systems in which artificial insemination is the predominant mode of breeding and in which comingling of herds is avoided—for example, in the European dairy industry. A recent survey conducted in Switzerland involving 1362 preputial samples from bulls and 60 abomasal fluid samples of aborted fetuses from beef and dairy herds revealed no *T. foetus*-positive finding (Bernasconi et al. 2014). However, also in areas assumed to be largely free of bovine tritrichomonosis, a reestablishment of the infection in herds, especially in beef herds farmed under extensive, pastoral systems, is possible as shown by findings from Spain (Mendoza-Ibarra et al. 2012; Mendoza-Ibarra et al. 2013). Knowledge on risk factors is important for the implementation of effective measures to control tritrichomonosis. Results from risk factor studies were used to model effects of vaccination against tritrichomonosis in beef herds. A number of potential herd-level risk factors were assessed, including “no. of cows,” ”no. of young bulls,” ”trichomonad testing yes/no,” ”no. of trichomonad tests,” ”shared grazing,” “previous diagnosis of trichomonosis,” or ”duration of breeding season” (Villarroel et al. 2004).

T. foetus isolated from cattle and cats and *T. suis* from pigs are genetically very similar or in case of *T. foetus* from cattle and *T. suis* even indistinguishable. Nevertheless, there is no evidence that there are links between life cycles of *T. foetus* in cattle and cats or between life cycles of feline or bovine *T. foetus* and porcine *T. suis*. Most likely these parasites have evolved separately, and despite their genetic similarity, *T. foetus* of bovine and feline origin and porcine *T. suis* show biological traits which differ considerably.

As stated in Sect. 14.1.2, tritrichomonosis is an almost exclusively venereal transmitted disease in cattle and affects predominantly adult animals (Sager et al. 2007; Ondrak 2016). *T. foetus* is transmitted during coitus, mainly from an infected bull to an uninfected dam or vice versa (Ondrak 2016). Single mating with an infected bull may result in a 95% infection rate among susceptible heifers (Parsonson et al. 1976), but in general a transmission rate of 30–70% is assumed (Bondurant 2005).

A mechanical transmission either by uninfected bulls or by contaminated equipment or cryopreserved semen seems to be possible, but the relative importance of these routes of transmission is minor (Ondrak 2016; Clark et al. 1977; Murname 1959; Goodger and Skirrow 1986; Blackshaw and Beattie 1955; Clark et al. 1971; Skirrow and BonDurant 1988).

The following risk factors for tritrichomonosis in individual animals have been identified:

- **Carrier state and age as a risk factor of infection**: Infection in bulls is reported to persist for more than 3 years and may persist for life (Rhyan et al. 1999; Campero et al. 1990; Flower et al. 1983; Bondurant 2005). Several studies established that the likelihood of bulls being infected seems to increase with age (Skirrow et al. 1985; McCool et al. 1988; BonDurant et al. 1990; Rae et al. 1999, 2004; Mendoza-Ibarra et al. 2012) (Table 14.1). In one experiment using bulls—3 to 6 years of age—all bulls more than 4 years old became infected after three to six services while only one of two young bulls, 2–3 years of age, became infected after nine services (Clark et al. 1974). Bulls less than 4 years of age are rarely carriers of *T. foetus* (BonDurant 1985; Perez et al. 1992; Ondrak 2016; Kimsey et al. 1980; Skirrow et al. 1985; BonDurant et al. 1990). The reason for this finding is not completely understood. Old bulls may have had a higher number of sexual contacts than young bulls. An often mentioned other possible reason is the more pronounced invaginations in the penile and preputial epithelium of older bulls—i.e., the crypts of these epithelia are becoming deeper and increase in number with the age of the bull (BonDurant 1985; Skirrow et al. 1985; McCool et al. 1988; Perez et al. 1992; Ondrak 2016). However, the hypothesis that anatomical changes are the cause for older bulls found to be infected more often was recently questioned because no age-related statistically significant differences were observed in the surface architecture of the penile and preputial epithelium of bulls (Ondrak 2016; Strickland et al. 2014).

  In female cattle, age seems not to be associated with the likelihood of infection; however, there is evidence that repeated exposure induces resistance to infection (Simmons and Laws 1957; Clark et al. 1986; Skirrow and BonDurant 1990b). While mature bulls seem to remain infected for life, most cows are able to clear the infection after a few months—usually after 1 to 3 months—rarely longer (Parsonson et al. 1974, 1976; Skirrow and BonDurant 1990b; Bondurant 2005). Several studies of infected cows indicate that the os cervix is the preferred site of multiplication and persistence (Skirrow and BonDurant 1990b). Initial multiplication of *T. foetus* after infection seems to be followed by a decline of parasite numbers until next estrus (Bartlett and Hammond 1945). The numbers of parasites present in the cervicovaginal mucus seem to fluctuate during the estrus cycle, and the largest numbers are seen a few days before estrus (Hammond and Bartlett 1945). In two infected heifers that have been followed over time after experimental infection, *T. foetus* was not always observed in vaginal mucus by microscopic examinations or culture isolation suggesting fluctuations in vaginal parasite concentration; however, only in one heifer, there was a coincidence between the detection of *T. foetus* and the time of estrus (Simmons and Laws 1957). Not all cows are able to clear infection. Carrier dams have been reported—e.g., two chronically infected dams were observed in one Australian study 16 and 22 months after initial infection (Alexander 1953). In a Californian dairy herd, infected cows were found positive 9 weeks after pregnancy or 63 days after parturition (Skirrow 1987; Goodger and Skirrow 1986). In a more recent study from Argentina, several

heifers remained infected up to 300 days after breeding which underlines the importance of these carrier state heifers for persistence of infection in affected herds (Mancebo et al. 1995).

- **Herd management practices:** The risk of bulls of being tested *T. foetus* positive increased when the number of bulls used per unit was higher than 10 or the bull-to-cow ratio per unit was lower than 1 to 25. The higher number of bulls and lower bull-to-cow ratios are typical management practices in large herds to increase conception rates (Rae et al. 2004). It was hypothesized that by these practices the number of potential sexual contacts per bull and the probability for an individual bull to become positive are increased (Rae et al. 2004).
- **Breed disposition:** The possibility of a breed predisposition was discussed based on study results suggesting a higher prevalence of infection in particular breeds (BonDurant et al. 1990; Perez et al. 1992; Rae et al. 1999, 2004; Skirrow et al. 1985; Abbitt and Meyerholz 1979). A number of epidemiological studies addressed this question (Table 14.2). It had been hypothesized that in *B. taurus indicus* bulls, due to their longer preputial length, the likelihood of being infected might be higher (BonDurant et al. 1990). Statistically significant differences in prevalence were observed when *B. taurus taurus* and *B. taurus indicus* or *B. taurus taurus*/*B. taurus indicus* crosses were compared; highest prevalences of infection were observed in *B. taurus taurus* (BonDurant et al. 1990). Another study in Costa Rica also found a strong association between the risk of positive findings and the *Bos taurus taurus* breed—as compared to *Bos taurus indicus* pure or hybrid breeds (Perez et al. 1992); an additional study also showed an increased risk in Angus, Charolais, Hereford, or Simmental breeds relative to *B. taurus indicus* (Rae et al. 2004) (Table 14.2). However, findings suggesting breed disposition should be interpreted with care; studies might have been biased by uneven study designs or by not paying enough attention to the differences in the way herds of particular breeds were operated (Ondrak 2016). It was hypothesized that prevalence differences could be due to the increased number of matings accomplished by *B. taurus taurus* bulls as compared to *B. taurus indicus* bulls in the same period of time, thus increasing the risk of exposure to infection (Perez et al. 1992). However, there are also some studies that did not observe differences in prevalence of infection between *B. taurus taurus* and *B. taurus indicus* (Dennett et al. 1974).

  Nevertheless, a breed disposition and genetically based predisposition should not be completely ruled out. Further studies are needed to elucidate the reason why some of the infected dams are becoming carrier of *T. foetus*—being still infected after pregnancy—while others immediately eliminate infection during the first 2 to 3 months after loss of the conceptus (Alexander 1953; Goodger and Skirrow 1986; Skirrow 1987; Mancebo et al. 1995). It is possible that like in other

Table 14.2 Risk factor studies in bovine tritrichomonosis

Country	Region	Type of herd or animal	No. of herds examined/ no. of herds positive (%)	No. of animals examined/no. of animals positive (%)	Diagnosis	Herd-level risk factors	Individual-level risk factors	Type of study	Reference
Argentina	Province Buenos Aires	Beef	42/173 (24)	NA	C (modified Plastridge medium, Tricoazul), all bulls per herd, three sequential tests	Rearing herds vs. full-cycle herds, $p = 0.052$; pregnancy rate in cows ≤ 90%, $p = 0.005$[a]; shared livestock with others, $p = 0.003$[a]; rotation of bulls, $p<0.05$; abortion, $p<0.05$; *T. foetus* reported in previous year, $p = 0.001$[a]	NA	Case-control	Mardones et al. (2008)
Argentina	Province La Pampa	Beef	194/3766 (5.15)	309/29178 (1.06)	C with modified Diamond's medium (MDM), all non-virgin bulls, twice a year	Seasonal effect: highest no. of positive findings in February (pre-breeding season), no statistics provided; spatial clustering in south of La Pampa, $p = 0.008$; coinfection, high-risk cluster cells for bovine genital campylobacteriosis were also high-risk cells for bovine tritrichomonosis, $p = 0.0014$	NA	Cross-sectional	Molina et al. (2013)

(continued)

Table 14.2 (continued)

Country	Region	Type of herd or animal	No. of herds examined/ no. of herds positive (%)	No. of animals examined/no. of animals positive (%)	Diagnosis	Herd-level risk factors	Individual-level risk factors	Type of study	Reference
Australia	Victoria River District	NA	27/41 (65.5)	81/1008 (8.0)	C (modified Plastridge medium)	NA	Tritrichomonosis infection rates varied significantly with age ($p<0.0001$), that is, increasing with age ($p<0.05$); no evidence of an increased likelihood of coinfection with *Campylobacter fetus*	Cross-sectional	McCool et al. (1988)
Costa Rica	NA	Dairy, mainly	10/63 (15.9)	14/225 (6.2)	C (InPouch TF)	NA	Bull in service: no vs. yes, $p = 0.02$[a]; age > 3 years, $p = 0.02$[a]; breed, *B. taurus taurus* vs. *B. taurus indicus*, $p = 0.02$[a]	Cross -sectional (results of pilot study not included)	Perez et al. (1992)

Spain	Asturias de la Montana	Beef	27/65 (41.5)	33/103 (32)	C (InPouch TF or thyoglycollate transport medium (TFTM) with modified Diamond medium (MDM))+PCR (Felleisen et al. 1998)	Increased number in repeat breeder cows, $p = 0.007$	Age >3 years, $p = 0.04$	Cross-sectional	Mendoza-Ibarra et al. (2012)
Spain	Asturiana de los Valles	Beef	12/229 (5.2)	13/327 (4.0)	C (InPouch TF)+PCR (Felleisen et al. 1998)	No statistical significant predictors	Age >3 years, $p<0.001$ (univariable)	Cross-sectional	Mendoza-Ibarra et al. (2013)
USA	Florida	Beef	17 (40.4)	119/1984 (6)	C	Herd size ≥500, $p = 0.004$[b]; bull-to-cow ratio = 1:<25, $p = 0.039$[b]	Age ≥5 years, $p = 0.022$[a]; breed, Angus, Charolais, Hereford, Simmental vs. *B. taurus indicus*; $p<0.031$[a]; herd management, number of bulls per group ≥10, $p = 0.002$[a], bull-to-cow ratio <1:25, $p = 0.03$[a]; no knowledge of farmer on tritrichomonosis, $p = 0.003$; geographical area, South Florida vs. North Florida, $p = 0.001$[a]	Cross-sectional	Rae et al. (2004)

(continued)

Table 14.2 (continued)

Country	Region	Type of herd or animal	No. of herds examined/ no. of herds positive (%)	No. of animals examined/no. of animals positive (%)	Diagnosis	Herd-level risk factors	Individual-level risk factors	Type of study	Reference
USA	Idaho	Beef	65/159 (40.9)	NA	dM or C (InPouch TF, modified Diamond medium (MDM))	Total cattle grazed on FS (US Forest Service) allotment >844, $p<0.05$; commingling on BLM (Bureau of Lands Management) allotment, FS (US Forest Service) allotment, or on any public land allotment, $p<0.05$	NA	Case-control	Gay et al. (1996)
USA	Wyoming	Beef	8/303+8 (2.6)	NA	C or PCR (three consecutive cell cultures or one PCR by an accredited diagnostic laboratory); herd status based on findings in the past 3 years	Allotments neighboring a positive herd(s), $p = 0.0003$; allotment type, open/public vs. private, $p = 0.003$; mingling with neighboring herd(s), $p = 0.026$	NA	Cross-sectional	Jin et al. (2014)

USA	California	Beef	9/57 (15.8)	30/729 (4.1)	C, modified Diamond medium (MDM)	NA	Age >3 years, $p<0.025$; breed *(B. taurus taurus* vs. *B. taurus indicus (Bi)* or *Bi*-hybrids), $p<0.001$)	Cross-sectional	BonDurant et al. (1990)
USA	Texas	Mainly non-virgin bulls, tested prior to interstate or intrastate commerce	NA	NA/NA; 1154 positive results/31202 tests (3.7)	C + real-time PCR	Spatial cluster in southeastern Texas identified ($p<0.001$)	Proportion of positive findings was highest in August (5.5%), no statistics provided	Cross-sectional	Szonyi et al. (2012)

NA not applicable, *C* culture

[a]Statistically significant in multivariate logistic regression

diseases there are genetic determinants influencing a predisposition to acquire infection and to develop immunity against the pathogen.

- **Other individual-level risk factors:** One study observed that the probability of positive findings is lower in bulls in service—i.e., in sexually active bulls (Perez et al. 1992) (Table 14.2). This is in accord with previous findings, which suggested that a depletion of the preputial *T. foetus* population might occur because of intense sexual activity. It also supports recommendations of a sexual rest of at least 1 to 2 weeks before sampling bulls in order to improve the likelihood of accurately identifying *T. foetus*-positive bulls (Clark et al. 1983a; BonDurant 1985; Yule et al. 1989a; Ondrak 2016). Variations in sexual activity between different seasons may also explain fluctuating differences in the proportion of positive findings in bulls during a year (Molina et al. 2013; Szonyi et al. 2012).

In addition, there are a number of herd-level risk factor studies—case-control and cross-sectional studies—that have been carried out to elucidate in more detail management practices and other factors increasing the risk of herds to acquire tritrichomonosis (Table 14.2) (Mardones et al. 2008; Molina et al. 2013; McCool et al. 1988; Perez et al. 1992; Mendoza-Ibarra et al. 2012, 2013; Rae et al. 2004; Gay et al. 1996; Jin et al. 2014; BonDurant et al. 1990; Szonyi et al. 2012). Many of the identified explanatory variables are related to predictors or risk factors in favor to increase the likelihood of venereal transmission of *T. foetus* in herds and between herds:

- **Transmission between herds:** Risk factors were identified which characterized the likelihood to acquire tritrichomonosis from other herds. "Allotments neighboring a positive herd(s)"; "allotment type, open/public vs. private"; and "mingling with neighboring herd(s)" were recently identified as risk factors in *T. foetus*-positive beef herds in Wyoming, USA (Jin et al. 2014). These findings confirm previous observations in beef herds from Idaho, which identified "commingling on BLM—Bureau of Lands Management—allotment; FS, US Forest Service, allotment; or on any public land allotment" as an important predictor of positive herds (Gay et al. 1996). Also in Argentina "shared livestock with others" was identified as a significant risk factor for herds testing positive, that is, having positive bulls (Mardones et al. 2008). In summary, these findings suggest that any type of mingling herds and also fence-line contact with other herds represent important risk factors and should be avoided by farmers (Jin et al. 2014).
- **Transmission within herds:** Other predictors characterized the likelihood of transmission within a herd. Obviously, herd size plays a role. A study on beef herds in Idaho, USA, identified "total cattle grazed on FS—US Forest Service—allotment more than 844" as a risk factor, and a study from Florida, USA, observed that herds with more than or equal to 500 cows had a higher risk of being positive (Gay et al. 1996; Rae et al. 2004). The latter study also identified particular herd management practices increasing the individual risk of bulls to test positive; therefore, most likely, herd size is a confounder explained by man-

agement practices in large herds in favor for *T. foetus* transmission, like "no. of bulls used per unit larger than or equal to 10" or "bull-to-cow ratio per unit smaller than 1 to 25" as discussed earlier (Rae et al. 2004). The management practice "rotation of bulls within a herd" has been identified in an Argentinian study to most likely favor to perpetuate transmission of infection within a herd (Mardones et al. 2008). The same may apply to an observation that among "rearing beef herds"—i.e., herds that rear cattle until the weight of 150–250 kg—the prevalence of positive herds was higher as compared to "full-cycle herds," herds with breeding, rearing, and fattening. This observation was explained by hypothesizing a higher proportion of reproductively active animals resulting in an increased spreading of disease in "rearing beef herds" compared to "full-cycle herds" (Mardones et al. 2008). The risk factors related to a potential perpetuation/acceleration of transmission inside herds cannot be easily used to give recommendations with respect to better management practices, because these risk factors are either unspecific or difficult to change.

Herd-level predictors, possibly related to effects of tritrichomonosis include infertility, early fetal death, and rarely, abortion. In epidemiological studies "pregnancy rate in cows higher than or equal to 90%" and reporting "abortion" were found associated with *T. foetus*-positive beef herds in Argentina (Mardones et al. 2008). An "increased number in repeat breeder cows" was identified as a predictor for positive herds in extensively managed beef cattle in Spain (Mendoza-Ibarra et al. 2012).

Additionally, in a few studies, spatial clustering of *T. foetus*-positive herds was observed, and in one of these studies, it was also observed that high-risk clusters for *T. foetus* correlated to high-risk clusters for bovine genital campylobacteriosis (Molina et al. 2013; Szonyi et al. 2012). The observed spatial clustering remained unexplained in these studies.

14.3.2 Epidemiology of Tritrichomonosis in Cats

Although the typical clinical sign in natural infection is chronic or intermittent diarrhea, for many cats, no diarrhea was reported in 6 months preceding diagnosis (Xenoulis et al. 2013; Kuehner et al. 2011). Chronic *T. foetus*-associated diarrhea in most cats is likely to resolve spontaneously within 2 years of onset, and chronic infection with *T. foetus*—without clinical signs—after resolution of diarrhea appears to be common (Foster et al. 2004). In experimentally infected kittens, *T. foetus* infections was long lasting and that, in later phases of the infection, the presence of *T. foetus* in feces was not always associated with clinical signs—such as abnormal consistency of feces (Gookin et al. 2001). Thus, in addition to disease-infected cats, cats with asymptomatic infection are able to transmit infection to other cats.

As mentioned in Sect. 14.1.1, feline *T. foetus* tolerates a broader pH range than *T. foetus* from cattle (Morin-Adeline et al. 2015a). Like for *T. foetus* from cattle, no encysted stages are known for *T. foetus* from cats. However, the formation of

pseudocysts, which may support survival in the environment, has been reported for *T. foetus* (Pereira-Neves and Benchimol 2009). In addition, it has been shown that feline *T. foetus* is able to survive outside of its host for at least 30 min on dry cat food and 180 min in drinking water or urine (Rosypal et al. 2012). Other studies showed that *T. foetus* can survive in cat feces for several hours or even days or up to at least 5 days in wet cat food (Hale et al. 2009; Van der Saag et al. 2011). Even the survival of a passage through the alimentary tract of slugs (*Limax maximus*, *Limacus flavus*) was demonstrated, suggesting that slugs could transmit *T. foetus* over short distance (Van der Saag et al. 2011). The predominant mode of infection is the fecal-oral route, and most likely a close contact of cats favors the spread of transmission.

Several epidemiological studies are reported, providing data on putative risk factors for *T. foetus* infection in individual cats or catteries, cat shelters, and breeding centers (Table 14.3). Most of the studies were small-scale studies, including only low numbers of individual cats and catteries, shelters, or breeding centers. Some of the studies were restricted to cats from shows or pedigree cats. Some studies were restricted to diarrheic cats or cats with a history of chronic diarrhea. Therefore, studies listed in Table 14.3 were stratified into (1) studies including only healthy or healthy and diseased cats and (2) studies including almost only diseased cats—i.e., cats with diarrhea or a history of diarrhea. In addition to studies listed in Table 14.3, a large number of case reports and small-scale studies are available—recently reviewed by Yao and Köster (2015)—which are not mentioned in the following because these studies provided no statistical evidence on putative risk factors. As indicated in Table 14.3, some studies were included after performing own statistical analyses based on data extracted from the reports.

Individual risk factors for tritrichomonosis in cats include**:**

- **Diarrhea, abnormal fecal consistency, and history of diarrhea.** In a number of studies including only healthy or healthy and diseased cats, it was observed that positivity for *T. foetus* was associated with a "previous history of diarrhea," "history of diarrhea in the past 6 months," "presence of chronic diarrhea," and "abnormal fecal consistency" (Table 14.3) (Kuehner et al. 2011; Tysnes et al. 2011; Doi et al. 2012). These findings confirm that *T. foetus* is an important cause of diarrhea in cats.

  The likelihood of a cat to test *T. foetus* positive was also increasing if there was a "history of another cat in the house with diarrhea in the past 6 months" or a "history of diarrhea in cattery in the past 6 months," which suggested that individual positive cats were only part of a larger problem in a cattery, cat shelter, or breeding center (Kuehner et al. 2011; Hosein et al. 2013).

- **Age.** With a few exceptions, it is now accepted that *T. foetus* associated with large bowel diarrhea is mainly a disease of young cats. Several studies revealed that cats about 1 year old or younger are more often *T. foetus* positive than elder cats (Table 14.3). These observations were made in studies including only healthy or healthy and diseased cats as well as in studies including almost only diseased cats (Profizi et al. 2013; Kuehner et al. 2011; Queen et al. 2012; Galián

Table 14.3 Risk factor studies in feline tritrichomonosis

Sampled animals	Country	Region	Type of animal	No. of catteries, shelters, or breeding centers examined/ no. of catteries positive (%)	No. of animals examined/ no. of animals positive (%)	Diagnosis	Cattery-level risk factors	Individual cat-level risk factors	Type of study	Remarks	Reference
Only healthy or healthy and diseased cats											
	Canada	Ontario	Cats sampled at a cat clinic (n=140), cat shows (n=55), and a humane society (n=46)	NA	14/241 (5.8)	C (InPouch TF), PCR for confirmation of culture positives		Attendance to cat shows, $p<0.05$ (23.6% from the show cats were positive); history of another cat in the house with diarrhea in the past 6 months, $p<0.01$; fed a raw food diet, $p<0.01$; >5 cats per house, $p<0.01$; purebred vs. mixed breed, $p<0.01$	Cross-sectional	No significant association between the presence of *T. foetus* and diarrhea at the time of sampling or having a history of diarrhea in the past 6 months; "attendance to cat shows" was the only significant variable in bivariable models	Hosein et al. (2013)

(continued)

Table 14.3 (continued)

Sampled animals	Country	Region	Type of animal	No. of catteries, shelters, or breeding centers examined/ no. of catteries positive (%)	No. of animals examined/ no. of animals positive (%)	Diagnosis	Cattery-level risk factors	Individual cat-level risk factors	Type of study	Remarks	Reference
	France	NA	140 cats cattery-housed pedigree cats, participating in international cat shows	18/117 (15.9)	20/140 (14.3)	C (InPouch TF) + Tricho-F/ Tricho-R-PCR (TRICHO-F and antisense primer TRICHO-R targeting the ITS1-5.8S rDNA-ITS2 region as previously described (Jongwutiwes et al. 2000); (Duboucher et al. 2006) + Seq (amplicon, cloned)		Age < 1 year, $p = 0.057$	Cross-sectional	No significant associations: size of cattery, type of food, vicinity of dogs	Profizi et al. (2013)

	Germany	NA	230 purebred cats	23/124 (15.7)	36/230 (18.5); C-positive: 29/230, PCR-positive: 28/230	C + PCR (Grahn et al. 2005) + Seq (Representative amplicons)		Abnormal fecal consistency, $p<0.001$; history of diarrhea in the past 6 months, $p = 0.027$; age ≤1 year old, $p<0.034$; Norwegian Forest (NFO) cats had a significantly higher prevalence of *T. foetus* infection than other breeds, $p< 0.001$; history of diarrhea in cattery in the past 6 months ($p = 0.01$)	Cross-sectional	Any	Kuehner et al. (2011)
	Japan	Hokkaido, Saitama	147 samples, also from cats with chronic diarrhea	NA	13/147 (8.8)	Cultivation (98 samples) + PCR (all samples)	NA	Presence of chronic diarrhea, $p<0.0035$	Cross-sectional	No significant differences with respect to age, breed, and whether cats were maintained indoors/outdoors between infected and uninfected cats (Fisher exact test)	Doi et al. (2012)

(continued)

Table 14.3 (continued)

Sampled animals	Country	Region	Type of animal	No. of catteries, shelters, or breeding centers examined/ no. of catteries positive (%)	No. of animals examined/ no. of animals positive (%)	Diagnosis	Cattery-level risk factors	Individual cat-level risk factors	Type of study	Remarks	Reference
	Norway	NA	52 clinically healthy cats participating in 3 cat shows	NA	11/21 (21)	Direct microscopy (n=39) + C (n=39) + nPCR (n=52), Seq of TFR3/TFR4 amplicons	NA	Own previous history of diarrhea, $p = 0.1$ (indicated as trend)	Cross-sectional, pilot study	Four samples positive for *Giardia* spp., one TF-*Cryptosporidium* spp. coinfection	Tysnes et al. (2011)
	USA	NA	173 cats with and without clinical signs of tritrichomonosis; 32 purebred (including 2 purebred-cross cats), 143 mixed breed cats	NA	17/173 (9.8)	C + confirmation by TFR3/ TFR4-PCR + Seq (amplicons)	NA	No statistical analysis provided in reference. Retrospective statistical analysis of presented data shows that purebred cats have a higher risk than mixed bred cats, Fisher exact test, $p<0.001$	Cross-sectional, pilot study	All positive cats had diarrhea. Positive cats were between 6 weeks and 12 yrs old, Negative cats 4 weeks to 13 yrs old. Positive cats often (53%) had concurrent infections (*Giardia* spp., *Cryptosporidium* spp., Coccidia, FIP)	Stockdale et al. (2009)

	USA	NA	117 cats from 89 catteries at an international cat show	28/89 (31.5)	36/117 (30.8); In detail, fecal smear (5/36), fecal culture in modified Diamond's medium (9/36), fecal culture in InPouch TF (20/36), or nPCR (34/36)	Fecal smear examination, C in modified Diamond's medium, C in InPouch TF medium, or nPCR amplification (Gookin et al. 2002)	Loose stools or diarrhea in any cats within the past 6 months, $P<0.052$; coinfection with coccidia, $P<0.059$; square feet of facility available per cat was low, $P<0.056$	NA	Cross-sectional	An association between *T. foetus* and *Giardia* spp. infection was not significant ($p = 0.075$). There were no differences in age or sex between uninfected cats and cats having *T. foetus* infection. The proximity of the cattery to within 0.5 miles of agricultural species, type of diet fed to cattery cats (commercial or home cooked), and source of water were not associated with risk of infection	Gookin et al. (2004)
	USA	California	Diarrheic (n=219) and control cats (n=54)	NA	10/223 (4.5)	C and/or PCR	NA	Age: mean age of positive cats 0.7 ± 0.3 years, mean age of negative cats 5.4 ± 5.0 years, $p<0.001$	Case-control	Any	Queen et al. (2012)
Almost only diseased cats											

(continued)

Table 14.3 (continued)

Sampled animals	Country	Region	Type of animal	No. of catteries, shelters, or breeding centers examined/ no. of catteries positive (%)	No. of animals examined/ no. of animals positive (%)	Diagnosis	Cattery-level risk factors	Individual cat-level risk factors	Type of study	Remarks	Reference
	Europe	NA	Cats submitted to a private veterinary laboratory due to diarrhea	NA	166/1840 (9.02)	TFR3/ TFR4-PCR	NA	No statistical analysis provided in publication. Results of retrospective analysis: age ≤1 years (65/628 positive), 2–15 years (33/755 positive), statistically significant difference, chi-square, $p<0.001$	Cross-sectional	Any	Galián et al. (2011a, 2011b)
	Germany and Austria	NA	31 cats (6 weeks–14 years old; 30 had diarrhea)	NA	6/31 (19.4%)	nPCR+Seq (Amplicon)	NA	Purebred cats, $p = 0.0153$	Cross-sectional, pilot study	Coinfection with *Giardia* (ELISA) likely in four of the *T. foetus*-positive cats, there was only one cat *Giardia* positive but TF negative	Steiner et al. (2007)

	Spain	NA	Cats from densely housed origins with a history of chronic diarrhea: family cats (*n*=15), breeding center cats (*n*=28), cat shelter cats (*n*=50); healthy cats: family cats (*n*=20), cat shelter cats (*n*=22)	3/4 (75); 50 cats were from 1 breeding center and 3 shelters	36/93 (38.7) diarrheic cats from cattery or family cats; 0/20 (0) healthy cats	C in modified Diamond's medium and/or TFR4/ TFR3-PCR	NA	Age ≤1 years, $p = 0.014$	Cross-sectional study, cats from densely housed origins with a history of chronic diarrhea and healthy cats	No difference between purebred (Persian) and mixed breed; female vs. male, $p = 0.085$	Arranz-Solis et al. (2016)
	UK	NA	111 naturally voided diarrheic feline fecal samples	NA	16/111 (14.4)	nPCR (Gookin et al. 2002)	NA	Age ≤ 1 years, $p = 0.0026$; pedigree cat, $p = 0.018$; Siamese and Bengal breed, $p = 0.0077$	Cross-sectional, pilot study	Any	Gunn-Moore et al. (2007)

(continued)

Table 14.3 (continued)

Sampled animals	Country	Region	Type of animal	No. of catteries, shelters, or breeding centers examined/ no. of catteries positive (%)	No. of animals examined/ no. of animals positive (%)	Diagnosis	Cattery-level risk factors	Individual cat-level risk factors	Type of study	Remarks	Reference
	UK	NA	Fecal samples from diarrheic cats submitted to a private veterinary laboratory	NA	205/1088 (18.8)	Real-time PCR (IDEXX 8-way PCR assay: 5.8S rDNA, AF339736)		Age: 6–12 months (29.4%) vs. >12 months (15.2%), $p<0.001$; pedigree cats (37.8%) vs. non-pedigree cats (6.0%), $p<0.001$; *T. foetus*-positive findings tended to be found in combinations with *Coronavirus*, *Clostridium perfringens*, and *Giardia* sp	Cross-sectional	<6-month-old cats had lower *T. foetus* prevalence than 6–12-month-old cats	Paris et al. (2014)

NA not applicable, *C* cultivation, *nPCR* nested PCR, *Seq* sequencing

et al. 2011a, b; Arranz-Solis et al. 2016; Gunn-Moore et al. 2007; Paris et al. 2014). These epidemiological findings confirm the results of follow-up studies of cats with *T. foetus* infections, which clearly showed that clinical signs spontaneously resolve within 2 years of onset and that it is difficult to detect *T. foetus* in cats after several weeks of infection (Gookin et al. 2001; Foster et al. 2004). Meta-analysis on available data confirmed this view (Yao and Köster 2015). The reasons why especially young cats are affected by *T. foetus* diarrhea are not sufficiently understood. Possible explanations are a more intense contact of kittens to their mothers or to larger numbers of other cats in breeding centers, private households, or cat shelters, which may favor transmission of *T. foetus* and may also influence the dose by which kittens become infected. Immunity against feline *T. foetus* is poorly understood; recently the possibility that parasite-specific secretory IgA mediates immunity has been discussed (Yao and Köster 2015). Histological examinations in infected cats revealed influx of lymphocytes, plasma cells, and neutrophils into the subepithelial lamina propria (Yaeger and Gookin 2005). The inflammatory response might not only be involved in pathogenesis but may also be involved in the control of infection (Tolbert and Gookin 2016).

- **Breed.** A number of studies identified an increased risk of infection in purebred, or pedigree cats, or particular breeds (Hosein et al. 2013; Stockdale et al. 2009; Steiner et al. 2007; Gunn-Moore et al. 2007; Paris et al. 2014). These observations remain largely unexplained. Most likely the conditions under which purebred or pedigree cats are reared are responsible for the increased risk. Purebred cats from breeding centers represent densely housed populations, and due to the frequent, close, and direct contact, the risk of infection might be higher than in mixed breed cats reared in less dense populations (Yao and Köster 2015; Arranz-Solis et al. 2016).
- **Other risk factors.** One other risk factor identified was "attendance to cat shows," which might be a confounding predictor; the predictor could be related to the increased risk of purebred or pedigree cats (Hosein et al. 2013). There is only a single study report on food-related predictors. In a Canadian study, "fed a raw food diet" has been identified as a risk factor (Hosein et al. 2013).
- **Other concurrent infections and impaired immune system.** In experimental *T. foetus* infection with cats which shed naturally *Cryptosporidium parvum* oocysts and Cryptosporidium non-infected cats those with *C. parvum* infection had an earlier onset, more severe diarrhea, and increased number of trichomonads on direct fecal examination, as compared to non-infected cats (Gookin et al. 2001). In epidemiological studies none of the concurrent infections examined—*Giardia* sp., *Cryptosporidium* sp., coccidia, *Clostridium perfringens*, feline infectious peritonitis, and *Coronavirus*—revealed a statistical association either to infection or disease on the individual animal level (Table 14.3). Nevertheless, it has been discussed whether *T. foetus* alone can cause clinical signs without an impaired or immature immune system, concurrent infection, or other factors resulting in alterations in the colonic microflora (Manning 2010).

In catteries, cat shelters, or breeding centers, *T. foetus* may cause outbreaks of persistent large bowel disease (Holliday et al. 2009). There is only a single study on risk factors associated with occurrence of trichomonosis in catteries (Table 14.3). Similar to the findings in individual cats, the analysis on the cattery level also identified an association between *T. foetus* and abnormal fecal consistency or diarrhea; "loose stools or diarrhea in any cats within the past 6 months" was significantly associated with *T. foetus*-positive findings (Gookin et al. 2004). A second putative risk factor identified in this study was related to the cat population density in catteries; if the "square feet of facility available per cat was low," this was associated with positive findings (Gookin et al. 2004). The observation that "coinfection with coccidia" was associated with *T. foetus*-positive findings may suggest that there are common risk factors in favor of mixed infections of coccidian parasites with *T. foetus* (Gookin et al. 2004). In epidemiological studies none of the concurrent infections examined revealed a statistical association either to infection or disease on the individual animal level.

14.3.3 Epidemiology of *T. gallinae*

As outlined in Sect. 14.2.1, *T. gallinae* affects a large number of avian species where it mainly parasitizes the oropharyngeal membranes—sinuses, mouth, throat, and esophagus—causing a disease characterized by greenish fluid and caseous lesions, of whitish-yellowish fibrinous material, on the oropharyngeal membranes (reviewed by Amin et al. (2010)).

T. gallinae infection seems not to be host species-specific. However, studies on parasite diversity suggest that there are subtypes more commonly found in certain bird species (Gerhold et al. 2008; Grabensteiner et al. 2010; Sansano-Maestre et al. 2009). *T. gallinae* is most common among domestic pigeons and wild doves, and these species may represent a reservoir also for other bird species. Accordingly, rock pigeons—*Columba liva*—were regarded as source for the worldwide distribution of *T. gallinae* (Stabler 1954; Harmon et al. 1987). Most pigeons harbor this protozoan but rarely show clinical disease (Stabler 1954). Hawks, falcons, and owls may become infected, most likely via predation of other infected birds (Rogers et al. 2016). In recent years, severe outbreaks of avian trichomonosis caused by *T. gallinae* have been recorded initially in wild finches, later in Passeriformes, canaries, and psittacines in Europe and North America since 2005 (reviewed by Amin et al. (2010)). Trichomonosis is also reported in several other bird species, including corvids in California, USA (Anderson et al. 2009). *T. gallinae* has a low tenacity in the environment and is regarded as unable to survive a gastric passage, and droppings of birds are regarded as free of *T. gallinae* (Stabler 1954). *T. gallinae* has no cyst stage. However, as mentioned for *T. foetus* (Sect. 14.1.1), the formation of pseudocysts has been reported (Tasca and De Carli 2003). The importance of pseudocysts in the transmission of *T. gallinae*—e.g., via

contaminated drinking water—is not sufficiently understood. Survival times in tap water and in carcasses are regarded as limited; a survival time of 8–48 h in carcasses has been reported (Erwin et al. 2000). The following important facts about transmission of *T. gallinae* need to be taken into account:

- **Transmission by crop milk or direct contact:** Prevalences in pigeons can be very high, and pigeons are regarded as endemically infected, often without clinical signs. Feeding on infectious crop milk is regarded as an important route of infection for nestlings (Stabler 1954). In other bird species, feeding nestlings by regurgitation might also be an important route of transmission. Close contact—e.g., during courtship—is also regarded as a way by which the infection is spread between adult birds (Stabler 1954). In Southeastern USA, trichomonosis was diagnosed more often in the spring and summer months than in autumn and winter months, which might be related to the times of courtship and feeding nestlings (Gerhold et al. 2007).
- **Transmission by drinking water:** It is believed that infection of turkeys and chickens is caused by *T. gallinae*-contaminated drinking water (Stabler 1954). Drinking water is also a likely source of infection for other bird species.
- **Effect of weather events on outbreaks of avian trichomonosis:** *T. gallinae*-associated finch mortality usually peaked in summer and autumn, but a correlation with climatic events has not been observed (Neimanis et al. 2010; Robinson et al. 2010; Lawson et al. 2011). A coincidence of the emergence of avian trichomonosis with high temperature and low rainfall has been reported (Simpson and Molenaar 2006; Bunbury et al. 2007). It has been hypothesized that as a consequence of the dry and hot weather, numbers and volumes of water sources decline, which may favor, due to higher densities of birds aggregating at limited water sources, the transmission of *T. gallinae* (Bunbury et al. 2007).
- **Transmission by predation:** Pigeons and doves may also serve as a source of infection for raptors (Boal et al. 1998; Krone et al. 2005). Although *T. gallinae* is a labile protozoan which does not remain viable for a prolonged period and is rapidly killed by desiccation, survival time seems to be long enough to be transmitted also to the nestlings of raptors (Krone et al. 2005).

14.3.4 Epidemiology of *T. gallinarum*

In contrast to *T. gallinae*, *T. gallinarum* is mainly found as a commensal in the intestine of many domestic bird species, including chicken, turkey, guinea fowl, duck, and goose (Friedhoff 1982; BonDurant and Honigberg 1994). As a parasite of the intestine, *T. gallinarum* is transmitted via consumption of contaminated food or drinking water. Cloacal as well as oral experimental infection of chickens and turkeys is possible.

14.4 Prevention

14.4.1 Prevention in Cattle

Due to the widespread use of artificial insemination, bovine tritrichomonosis has almost disappeared in dairy cattle industries, like in Western European countries, in the USA, or in Canada. However, the disease is still present in many areas of the world were cattle are raised on pastures and natural mating is used. Prevention and control measures are based on the distinctive epidemiologic features of bovine tritrichomonosis, a sexually transmitted disease where infected bulls are asymptomatic carriers and represent a permanent source of infection while in heifers and cows infection is typically transient (Clark et al. 1971, 1974; Parsonson et al. 1974; Skirrow and BonDurant 1990b).

Bovine tritrichomonosis belongs to the OIE-listed diseases (http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2017; accessed 22. Febr. 2017). *T. foetus* as a causative agent of this venereal transmitted disease may be present in semen if the semen has been contaminated with preputial fluid during manual collection (Bondurant 2005). Recommendations for the importation of cattle and bulls for breeding can be found in the *Terrestrial Animal Health Code*. In particular, emphasis has been placed in the measures applied to bull semen donor health status in order to avoid dissemination and transmission of the disease (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_trichomonosis.htm; accessed 22. Febr. 2017).

In dairy herds and in some beef herds, artificial insemination is a very useful measure to reduce and eliminate infection. For bulls destined for artificial insemination, quarantine and periodic testing are required. Also, in semen trade, it is of great value to know the country of origin, the reproductive history of the bull, and the tests performed by the artificial insemination center (Eaglesome and Garcia 1997). In some areas of the world, as in the EU, specific regulations are applied to bovine semen trade and to regulate the sanitary conditions of the collection center and the animals. Specifically, bulls selected for entry into artificial insemination should be tested in quarantine before admission to the center, and regular testing of animals in service is included as basic measures to avoid the presence and dissemination of bovine tritrichomonosis (European Directive 88/407/EEC of 14th June 1988 and European Directive 2003/43/EC of 26th May 2003).

In other countries, different policies have been established to control the infection. In the USA, state regulations have endeavored to control the endemic disease as to minimize economic losses by testing bulls. Only the importation of *T. foetus*-free bulls is permitted for reproductive purposes while positive bulls are culled (Yao 2013). In the province of La Pampa, Argentina, with a bovine census close to four million heads, more than 80% of bulls are tested twice each year, and positive bulls were culled. By this measure, a significant decrease in the number of infected herds and animals has been achieved in this region in the last 10 years (Fort et al. 2016). Recently, an online tool has been developed in the USA—Trich CONSULT; www.trichconsult.org, accessed 22. Febr. 2017—that uses a series of questions to assess

the *T. foetus* status and management practices of a herd. Based on the responses to the questions, this page provides feedback to users allowing them to evaluate the importance of implementing suggested control strategies (Ondrak 2016).

At present, a consensus exists concerning the most relevant measures as to how to prevent and control bovine tritrichomonosis (reviewed in Ball et al. (1987), McCool et al. (1988), Bondurant (2005), Rae and Crews (2006), Campero and Gottstein (2007), Yao (2013), Ondrak (2016)).

In herds where it is not possible to introduce artificial insemination and where natural mating is the normal practice, as is the case in many regions where extensive beef cattle are raised, the following measures to prevent the entrance of the infection are recommended.

- **Quarantine and testing of replacement bulls.** Replacement should be done by virgin animals or bulls acquired only from disease-free herds with records of excellence in reproductive performance. All the bulls must be tested during quarantine before entrance in the herd. If the bull comes from a tritrichomonosis-free herd, the analysis of two samples of preputial smegma with 1–2 weeks of interval during the quarantine is recommended. Three or more samples must be analyzed if bulls are provided from an area where tritrichomonosis is known to be endemic (Campero and Gottstein 2007; Yao 2013; Ondrak 2016). The measure includes the prohibition of the use of communal, shared, or rented bulls, unless their herd of origin and individual health status are known.
- **Avoid communal pastures and keep fences in good conditions.** These measures will help to control some of the two most important risk factors influencing disease transmission (Gay et al. 1996; Mardones et al. 2008; Jin et al. 2014).
- **No introduction of cows or heifers of unknown health status during the breeding season.** Similar to bulls, heifers and cows must be acquired only from disease-free herds with records of excellence in reproductive performance. In Argentina several heifers were found infected up to 300 days after breeding; such heifers may represent carrier cows able to introduce infection into naïve herds (Mancebo et al. 1995).

In addition to the preventive measures outlined in Sect. 14.3, the following control measures are recommended to reduce the impact and eliminate the disease in case that bovine tritrichomonosis has been diagnosed in a herd or the herd is located in a tritrichomonosis endemic area:

- **Testing of bulls before the breeding season and culling of infected bulls.** Efforts to control the disease focus on using diagnostic tests with a high sensitivity, low cost, and time efficacy. It is recommended to sample the animal twice or three times before the breeding season and every time new bulls are introduced into herds (Bondurant 2005; Campero and Gottstein 2007; Yao 2013). Once new cases are not detected, annual testing is recommended to verify the non-infected status of the herd. However, testing and culling policies alone, although effective in improving reproductive efficiency, do not allow the elimination of the disease

since other putative risk factors associated with the disease are usually present in the management of beef herds (Yao 2013; Collantes-Fernandez et al. 2014).

- **Average age reduction of bulls and replacement of all bulls after four breeding seasons.** Replacement with negative-tested young bulls reduces the prevalence since 3-year-old bulls seem not to be as susceptible as older bulls in natural service (Clark et al. 1971; Christensen et al. 1977). A strong association of infection rate with age has been reported in several studies (Rae et al. 1999; Mendoza-Ibarra et al. 2012).
- **Pregnancy examination.** It is mandatory to know the reproductive performance and the magnitude of the infertility problem in the herd. All open and aborted cows should be culled or segregated in high-risk sub-herds or groups (Campero and Gottstein 2007).
- **Use of commercial vaccines.** In the presence of a high prevalence of infection in an area, vaccination of all heifers and cows is a good measure to improve reproductive efficiency especially when risk factors associated with infection cannot be avoided—e.g., the use of communal pastures. Commercially available vaccines in the Americas offer an improvement in reproductive efficacy (Kvasnicka et al. 1989, 1992).
- **Segregation of the herd in low- and high-risk sub-herds or groups.** In the low-risk sub-herds, only dams that have recently calved are pregnant for more than 5 months, and virgin females must be included. These must be serviced preferably by virgin bulls or by bulls with two negative examinations and coming from negative herds. In order to follow the effect of the infection, the same group of females should be serviced with the same male until the disease is controlled (Campero and Gottstein 2007).
- **Limiting of breeding season.** The breeding season should be restricted to less than 4 months to reduce the duration of the possible transmission period as much as possible. In addition, with a shortened breeding season, it becomes easier to monitor reproductive performance.

With respect to vaccination against bovine tritrichomonosis, the main objective is to eliminate a *T. foetus* infection from the reproductive tract before fetal loss occurs without necessarily avoiding parasite colonization of the epithelium during the first 40 days post-infection (BonDurant et al. 1993; Bondurant 2005). The mucosal immune response in the genital area seems to be the main protective mechanism which is characterized by a local response with the presence of IgA and IgG1 and a mild systemic response characterized by the presence of IgG2 and IgG1 (Skirrow and BonDurant 1990a; Anderson et al. 1996; Corbeil et al. 2008). As a rule, cows immunized with *T. foetus* have a humoral response pattern similar to that described for natural infections (Herr et al. 1991; BonDurant et al. 1993). However, genital IgA levels appear to depend on the type of antigen, adjuvant, and route of administration employed.

As to our knowledge, commercial vaccines against bovine tritrichomonosis exist only in the Americas but not in Europe. One available inactivated vaccine is prepared from whole organisms and can be acquired in a monovalent formulation—Trich Guard®—but also in a polyvalent formulation combined with *Campylobacter foetus*

subspecies venerealis and *Leptospira*, Trich Guard V5-L®. In addition, in Argentina, an alternative inactivated vaccine—Tricovac, Tandil Biological Laboratory—containing an oily adjuvant with a concentration of 5×10^7 trophozoites of *T. foetus* is available.

In several studies on heifers using whole-parasite-based vaccines, a reduction in the number of infected females, a shorter time of genital infection, and a higher percentage of pregnant females in comparison with control animals have been reported (Kvasnicka et al. 1989, 1992; Herr et al. 1991; Gault et al. 1995; Anderson et al. 1996; Cobo et al. 2002, 2004). In addition, a lower number of services, 1.44 vs. 1.73 in non-vaccinated animals, $p = 0.16$; higher percentage of pregnant animals at the first service, 66.7 vs. 33.3% pregnancies, $p < 0.05$; and a reduction of embryo/fetal losses of 56.4% were observed (Kvasnicka et al. 1992; Hudson et al. 1993).

Subunit vaccines have also been developed (Clark et al. 1983b; BonDurant et al. 1993; Corbeil et al. 1998). A trial with an experimental vaccine formulated with membrane antigens of *T. foetus* was conducted; cows were immunized with this vaccine and subsequently challenged by the vaginal route (Campero et al. 1999). The vaccine used was able to generate a specific humoral immune response and shorten the period of infection in the vaccinated animals compared to the controls. A greater efficacy of a *T. foetus* membrane vaccine compared to a whole cell vaccine was observed; the animals had a shorter duration of infection, a higher pregnancy rate, and a lower rate of fetal mortality (Cobo et al. 2002). Additional work has been done to identify *T. foetus* surface antigens such as TF1.17 and TF190 (Voyich et al. 2001). The application of the former in experimentally challenged heifers evidenced a rapid shortening of infection and a specific IgA production in genital secretions (Anderson et al. 1996; Corbeil et al. 1998).

Vaccine-induced immunity to *T. foetus* has not been studied in depth in bulls and is only mentioned in some earlier studies (Clark et al. 1983b, 1984; Soto and Parma 1989; Campero et al. 1990; Herr et al. 1991). In bulls older than 5 years, the whole cell vaccine lacked a preventive or curative effect (Clark et al. 1983b). Therefore, commercial Trich Guard® vaccine is not indicated for bulls (Herr et al. 1991; BonDurant 1997).

14.4.2 Prevention in Other Animals

Prevention of feline trichomonosis is based on interrupting the fecal-oral route of transmission, particularly in catteries, shelters, and other dense cat populations. Since the viability of the parasite in the environment is limited, strict cleaning and disinfection measures are sensible measures to be implemented (Hale et al. 2009). Additionally, contamination of food and water by *T. foetus* is also possible; consequently, regular replacement of water and food and cleaning and disinfection of watering troughs and food bowls should be recommended. Due to the suggested role of some invertebrates to function as mechanical vectors, the avoidance of their presence in the litter box area and food and drinking areas may help to prevent infection

transmission (Van der Saag et al. 2011). Finally, limiting contact between infected and non-infected cats will help to interrupt transmission of *T. foetus* in the population.

Measures to prevent *T. gallinae* outbreaks in wild as well as in captive birds are focused on actions to reduce sources of infection. One of the major aims would be to prevent attracting birds to feeding places. If feeding is necessary, such places should fulfill minimum requirements with regard to sanitary conditions, like regular changing of food and disinfection as suggested in a recent review (Amin et al. 2014). Since *T. gallinae* seems to require a wet environment to persist, drying of buildings and housing facilities following washing will support to control trichomonad infections. The prevention of the infection of prey birds, like pigeons nesting near urban areas, is necessary to prevent infection of predator birds. Due to the loss of habitat, predators have replaced their traditional prey mainly by urban pigeons (Boal et al. 1998; Höfle et al. 2000; Estes and Mannan 2003; Krone et al. 2005).

14.5 Treatment

14.5.1 In Cattle

In the past various imidazoles were used to treat bulls, but none was both safe and effective, and drug resistant strains were found (reviewed in Bondurant (2005), Rae and Crews (2006)). Specifically, ipronidazole is probably the most effective drug, but due to its low pH, it frequently causes sterile abscesses at the injection sites, and resistances have also been observed (Skirrow et al. 1985). A systemic treatment using drugs like metronidazole or dimetridazole produces adverse side effects and resistant populations of trichomonads (Campero et al. 1987). Currently, there is no approved treatment for cattle infected with *T. foetus* because of concerns regarding toxic residues in meat (BonDurant 1997).

14.5.2 In Cats

Therapies traditionally used for treatment of protozoa are not successful for feline trichomonosis (reviewed in Manning (2010), Yao and Köster (2015)). Currently, ronidazole has been the most effective drug used to date and is recommended at 20–30 mg/kg orally once daily for 14 days (Gookin et al. 2006). Relapse of diarrhea is common, but cats can continue to carry the organism, and resistant strains of *T. foetus* to ronidazole have also been documented (Gookin et al. 2010a). In addition, neurological toxicity in cats treated with ronidazole in the range of 30–50 mg/kg has been reported (Rosado et al. 2007). It is therefore important that owners are informed of the potential side effects. Ronidazole is not registered for veterinary use, and informed consent is necessary prior to its use in cats, and it should only be prescribed in confirmed cases.

14.5.3 In Other Animals

The drugs of choice for the treatment of avian trichomonosis are nitroimidazoles (metronidazole, dimetridazole, ronidazole, and carnidazole) (reviewed by Amin et al. (2014)). However, subtherapeutic dosing and prophylactic use of these drugs against trichomonosis have resulted in emergence of resistant strains of *T. gallinae* (Franssen and Lumeij 1992; Munoz et al. 1998). In wild birds, treatment is not a practical approach and generally not considered an option due to the way of application (Cole and Friend 1999). These drugs can be only used in non-food-producing birds by veterinary prescription.

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