Laboratory detection of *Clostridium difficile* in animals: a review

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Introduction

Clostridium difficile is a Gram positive anaerobic, sporogenic bacterium that forms part of the intestinal microbiota. As all pathogens of the *Clostridium* genus, *C. difficile* is also characterized by the fact that only toxigenic strains cause the disease. *C. difficile* can produce three toxins: toxin A (TcdA; 308 kDa), toxin B (TcdB; 207 kDa), which are the main virulence factors, and binary toxin (CDT), which is supposed to be an additional virulence factor, though its role in disease development is yet to be elucidated (Davies et al., 2011). Toxigenic strains can produce all three toxins (A+B+CDT+), only toxins A and B (A+B+CDT–), only toxin B and binary toxin (A–B+CDT+) or just one toxin (A–B+CDT–, A–B–CDT+) (Rupnik and Janežič, 2016). Recently, Monot et al. (2015) also described a strain producing only toxin A and being free of toxin B (A+B–), which could influence the choice of diagnostic tests.

Toxins TcdA and TcdB belong to the family of large clostridial toxins (LCTs). Both toxins are cytotoxic; in addition, TcdA is also enterotoxic (Just et al., 2000). The main consequences of TcdA activity are inflammatory changes in the intestinal wall and the collapse of close contacts between cells, resulting in diarrhoea and haemorrhagic necrosis. TcdB is responsible for the formation of ulcers and yellow-white coatings (pseudomembranes) on the colon wall (Voth and Ballard, 2005). CDT binary toxin, classified as clostridium binary toxins, is adenosine diphosphate-ribosyltransferase and is cytotoxic (Perelle et al., 1997). CDT was initially attributed to animal strains, though isolates producing CDT were recently detected in humans, and were related to a more severe disease and higher mortality (Gerding et al., 2014).

The genes for toxin A (*tcdA*) and B (*tcdB*) and three additional genes (*tcdR*, *tcdE*, *tcdC*) are located in the pathogenicity locus (PaLoc; 19.6 kbp) on the chromosome, and has the characteristics of a mobile genetic element. Non-toxigenic strains have a 115 bp sequence at the site of the PaLoc region (Hammond and Johnson, 1995). Depending on the differences in this locus, the strains are divided into 34 toxinotypes (Rupnik and Janežič, 2016). The expression of the *tcdA* and *tcdB* genes and the formation of TcdA and TcdB occurs in the late logarithmic and stationary stages of cell growth in response to various environmental signals (temperature, presence of glucose,

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amino acids, antibiotics and butyric acid) leading to various physiological changes in the cell and development of the disease (Voth and Ballard, 2005).

CDT encodes two genes: *cdtA* for the enzyme toxin subunit and *cdtB* for the transport toxin subunit, which are located on the chromosome (Perelle et al., 1997). *C. difficile* strains can thus be free of CDT genes, have a complete (4.3 kbp) or shortened CDT sequence - pseudogenes *cdtA* in *cdtB* (2.3 kbp), which do not lead to the expression of a functional CDT (Gerič Stare et al., 2007). Carter et al. (2007) discovered that the functional *cdt* locus (CdtLoc) contained a third gene called *cdtR,* which most likely encoded a response regulator important for optimal expression of binary toxin.

C. difficile is the most common cause of hospital intestinal infections (CDI) in humans. Because of the use of antibiotics, cytostatic treatment or surgery, normal intestinal microbiota is affected, allowing spore germination and consequent multiplication of *C. difficile* (Johnson and Gerding, 1998). CDIs cause mild diarrhoea, haemorrhagic colitis or severe pseudomembranous colitis and intestinal perforation (Bartlett et al., 1978). Community-acquired CDI has recently increased in people who have not been hospitalized, have not been treated with antibiotics, and have no other risk factors for the development of the disease (Pituch, 2009; Khanna et al., 2012). In these infections, PCR-ribotype 078/toxinotype V is frequently present, which is one of the most common types in different animal species (Hensgens et al., 2012).

C. difficile has been detected in various domestic and wild animals (horses, cattle, dogs, cats, ostriches, pigs, poultry, elephants, small ruminants…). Its role is that of a commensal or pathogenic bacteria in the digestive tract. *C. difficile* causes enterocolitis and diarrhoea mainly in neonatal piglets, calves and foals, while in elderly animals, the disease is most often associated with antibiotic treatment (Frazier et al. 1993; Baverud et al., 2003; Bojesen et al., 2006; Songer and Anderson, 2006; Keel et al., 2007; Clooten et al., 2008; Pirš et al., 2008; Simango and Mwakurudza, 2008; Koene et al., 2012; Knight and Riley, 2013; Silva et al., 2014). Piglets with *C. difficile* related diarrhoea can be underweighted and have an extended weaning time. Furthermore, Squire and Riley (2013) reported a 14% monthly mortality rate in piglets. Young animals are often clinically healthy carriers of *C. difficile;* however, colonisation rates decrease with age (Weese et al., 2010; Costa et al., 2011, Rodriguez et al., 2012). It has been demonstrated that there is no difference in the presence of *C. difficile* in piglets with and without diarrhoea, whereas the disease caused by *C. difficile* in poultry has not yet been described, and it appears that poultry is merely a bacterial reservoir (Zidarič et al., 2008; Alvarez-Perez et al., 2009).

Identical genotypes detected in animals, humans and food suggest the possibility of interspecies transmission of *C. difficile* (Jhung et al., 2008; Janežič et al., 2012). Animals could potentially represent a bacterial reservoir for human infections, especially in cases of community-acquired infections. In addition, *C. difficile* has been detected in food: in raw meat (pork, beef, chicken and turkey meat), prepared meat products, fish, shrimp, raw vegetables and prepared salads (Rupnik and Songer, 2010; Metcalf et al., 2011).

Laboratory methods for *Clostridium difficile* detection

Several methods are described for the detection of *C. difficile* in faecal samples. Most have been validated for diagnostics in humans and could theoretically be used in veterinary medicine. The

methods used in human medicine are described and critically evaluated for use in veterinary medicine below.

Toxigenic culture

Laboratory diagnostics is based on toxigenic culture involving the isolation of the bacterium from faeces and further *in vitro* demonstration of toxins with a cytotoxicity test or enzyme immunoassay (EIA) (Delmee et al., 2005). Toxigenic culture is the most sensitive among the described methods (sensitivity of 94-100%), but is time consuming and requires experienced personnel (Delmee, 2001; Cohen et al., 2010). Selective medium, i.e. fructose agar with cycloserine and cefoxitin (CCFA), is most frequently used for isolation (George et al., 1979) even though there are also other commercially available media, e.g. *C. difficile* agar with norfloxacin and moxalactam or chromogenic media Chrom ID *C. difficile* (BioMerieux, France) (Aspinall and Hutchinson, 1992). The media may contain various supplements that improve spore germination or enrich the medium (horse or sheep blood, egg yolk, lysozyme, cysteine, sodium taurocholate, etc.) or a colour changing indicator when *C. difficile* appears (CDSA agar with neutral red indicator; Becton Dickinson, USA) (Marler et al., 1992; Wilcox et al., 2000). The selective medium is incubated for 48 hours in an anaerobic atmosphere at 37 °C. The sensitivity of the method is increased using heat (80 °C) or alcohol shock before inoculating the sample (the same volume of faeces and ethanol is mixed and incubated for 30 minutes to one hour) (Borriello and Honour, 1981). Arroyo et al. (2005) compared various bacterial isolation procedures. The most efficient was enrichment selective broth (fructose broth with cycloserine and cefoxitine; CCFB) with the addition of 0.1% sodium taurocholate, which allowed better spore germination (Wilson et al., 1982), in combination with alcoholic shock followed by isolation *C. difficile* on selective plates. Hink et al. (2013) published the first study comparing several media and methods for *C. difficile* isolation from faeces and rectal swabs. In both cases the best combination was heat shock (heating for 10 minutes at 80 °C), followed by inoculation of the sample into mannitol broth with cycloserine, cefoxitin, taurocholate, cysteine and lysozyme, and then after seven days isolation of *C. difficile* on TSA II (tryptic soy agar) with 5% sheep blood. The use of enrichment broth improves the sensitivity of the method by about 20%, but the procedure is prolonged to about nine days (Tenover et al., 2011).

Recent studies compared chromogenic agar Chrom ID *C. difficile* (BioMerieux, France) with other selective agars and reported that black colonies could be observed after 24h incubation, though sensitivity significantly increased after 48h incubation. Furthermore, chromogenic agar yields a higher recovery of *C. difficile* than other selective agars, with a sensitivity of 100% and recovery of 94% (Carson et al., 2013; Han et al., 2014; Yang et al., 2014).

Identification of *C. difficile* colonies is based on typical morphology, yellow-green fluorescence under UV light, characteristic horse manure odour, gas liquid chromatography of colonies (evidence of butyric and iso-caproic acid) or detection of proline aminopeptidase with discs (Levett, 1984; Fedorko and Williams, 1997). The use of biochemical tests and antigenic latex agglutination usually does not give reliable results (Tenover et al., 2011). In recent years, laboratories with suitable equipment prefer identification of *C. difficile* with MALDI-TOF, which is reliable, fast and cheap (excluding initial costs for the machine) (Kim et al., 2016).

Cell cytotoxicity test

The reference method (″gold standard″) is still a cytotoxicity test for

the detection of toxin B, where cytopathic effects (cell rounding) are observed, though some authors prefer toxigenic culture as a reference method due its higher sensitivity (Cohen et al., 2010; Crobach et al., 2016). The most commonly used are Vero, Hep2, CHO or HeLa cells, with Vero cells being most sensitive. In the cytotoxicity test, the toxin is confirmed by neutralization with specific antibodies (Chang et al., 1978). The test is effective but has its shortcomings: lack of standardization, long turnaround time of 24 to 48 hours and need for experienced staff and maintenance of cell cultures; in addition, rapid decay or small amount of toxin in the sample hamper visible cytopathic effects, which leads to false negative results (Freeman and Wilcox, 2003; Delmee et al., 2005). On the other hand, false positive results may occur in neutralization with antibodies that can neutralize *Clostridium sordellii* lethal toxin (O'Connor et al., 2001). The cytotoxicity test is highly specific (99–100%) and has a good positive predictive value (PPV) (93–100%), while the sensitivity is lower (50–85%) compared to the toxicity culture (Stamper et al., 2009; Peterson et al., 2011).

Serological methods

Several commercial EIA tests are available for the detection of TcdB and/or TcdA toxins in faeces, though these are less sensitive than the cytotoxicity test (Barbut et al., 1993; Planche et al., 2008, Chand and Sod, 2011). The data about reliability of EIA tests vary; some studies report sensitivity of less than 50% and rarely over 90%. Additionally, low PPV is also a concern, especially in a low prevalence population. Meanwhile, specificity (88–100%) and negative predictive value (NPV) (83–99%) are satisfactory in most cases (Crobrach et al., 2016). In human medicine, EIA tests are widely used for screening, but the use of tests capable of detecting the presence of both toxins is necessary (Johnson et al., 2001). Despite their low cost and easy and rapid performance (results are obtained in 2–4 hours), recent studies have discouraged the use of EIAs as stand-alone tests (Eastwood et al., 2009; Cohen et al., 2010; Carroll and Loeffelholz, 2011; Boyanton et al., 2012). Commercially available tests are either EIA in microwell plates or membrane immune-chromatographic/lateral flow membrane EIA.

The EIA can also prove the presence of glutamate dehydrogenase enzyme (GDH), which is a specific *C. difficile* antigen, but does not distinguish toxigenic strains from non-toxigenic (Zheng et al., 2004). GDH EIA tests are characterized by high NPVs, therefore they are mostly used as the first screening test in multi-stage algorithms for the diagnosis of *C. difficile* infections (Reller et al., 2007). Their sensitivity for toxin detection is higher than in EIA tests, although this varies by manufacturer (78–100%) (Crobrach et al., 2016), while PPV is low (Shetty et al., 2011). In addition, the presence of a particular PCR-ribotype may reduce the sensitivity of the test, which raises concerns over its usefulness (Tenover et al., 2010).

Nucleic acid amplification tests (NAATs)

Over the past two decades, molecular methods such as PCR have been developed. These have reduced the need for experimental antibiotic treatment and consequently long-term hospitalization. Initially, molecular methods were limited to detection of the 16S rRNA gene and could not be used for differentiation between toxigenic and non-toxigenic strains. Subsequently developed PCR protocols included the detection of genes encoding toxins A and B (Gumerlock et al., 1993; Kato et al., 1993: Kuhl et al., 1993). Real-time PCR (rtPCR) for the detection of toxin genes is a faster and more sensitive, yet more expensive alternative.

Belanger et al. (2003) first described the use of rtPCR for the detection of *tcdA* and *tcdB* genes using molecular beacons on the SmartCycler (Cepheid, USA), but the *tcdB* genes in variant toxinotypes (III, IV, VI) were not detected. Later, several rtPCR protocols were published for the rapid detection of *C. difficile* in faeces, amplifying different target genes, using different chemistry and adapted for different thermocylcers. Compared with toxigenic culture as a gold standard, the sensitivity of in-house rtPCR tests ranges from 83 to 100%, the specificity is between 88 and 99%, PPV 66-94% and NPV 96-100% (Crobach et al., 2009).

Several commercial tests are available for the detection of toxigenic *C. difficile* directly in faeces, which are intended and tested for human use. At least 11 commercial NAATs are approved by the US Food and Drug Administration (i.e. BD GenOhm, ProDesse ProGastro Cd, Cepheid GeneXpert, Meridian Illumigene, etc.). However, specific equipment is needed to perform these tests and the cost per sample is much higher than for EIA, which are the major disadvantages for laboratories already equipped for molecular diagnostics (Martinez-Melendez et al., 2017). Most commercial NAATs are based on rtPCR, while some use isothermal helicase-dependent amplification with detection on an array or microparticle-based microarrays (Eckert et al., 2014). Several studies have been published to compare these tests with other methods. If the toxigenic culture was taken as a gold standard, the tests had a sensitivity of 62–100%, specificity of 87–100%, PPV 68–100% and NPV 95–100% (Martinez-Melendez et al., 2017).

One of the molecular approaches approved by the FDA is the loop-mediated isothermal amplification method (LAMP). The method is fast (1h) and its sensitivity, specificity and NPV are comparable to commercial rtPCR tests, while PPV is lower (83–92%) (Boyanton et al., 2012). Its disadvantage is that it only detects the *tcdA* gene, so false negative results for variant *C. difficile* types is observed (Lalande et al., 2011).

Several authors have warned of the excessive sensitivity of NAATs, since the positive result does not necessarily reflect the actual *C. difficile* disease, but only the presence of *C. difficile* (Polage et al., 2015). Peterson and Robicsek (2009) therefore recommend testing only those patients meeting the criteria for recurrent episodes of diarrhoea, and the clinical picture of the subject should be considered when evaluating the results of the diagnostic method (Dubberke et al., 2011). Moreover, NAATs do not prove the presence of toxins, only genes; therefore, it is not possible to know whether the gene is expressed and the toxin produced (*i.e.* clinical specificity) (Carroll and Loeffelholz, 2011).

Testing algorithms

Currently available diagnostic methods are either quick and less sensitive/specific or sensitive/specific and long-lasting (Tenover et al., 2011). Therefore, it is recommended to use that two-step algorithms for the diagnosis of *C. difficile* infection in humans (Carroll, 2011). The first screening test should be sensitive with a high NPV (GDH EIA, NAATs), and positive samples are then confirmed by confirmation test (toxin EIA, toxigenic culture) (Crobach et al., 2016).

Suitability of methods for the detection of *Clostridium difficile* in animals

All methods described above are potentially useful in veterinary medicine, though all have been developed and adapted for human use, with the exception of toxigenic culture for *C. difficile* isolation from faeces. In faecal samples from animals, there are often few vegetative cells and a higher number of spores;

therefore, pre-use of the enrichment selective medium is recommended, which prolongs the turnaround time. Thitaram et al. (2011) compared two isolation protocols on swine and cattle faeces samples: a single alcoholic shock after enrichment in a selective broth and a double alcoholic shock before and after enrichment. In both cases, CCFA and TSA media were used after enrichment. Double alcohol shock proved to work better for swine samples, while single alcohol shock was more suitable for bovine samples. The choice of medium had a minor impact, but it is nevertheless recommended to use *C. difficile* selective medium as it was easier to obtain a pure *C. difficile* culture. Avberšek et al. (2013) described an optimal cultivation method by implementing broth enrichment (CCFB with taurocholate) for seven days with a previous alcohol shock and subsequent isolation of *C. difficile* on selective agar (CCFA).

Initially, the use of EIA predominated in the veterinary medicine, but tests were only validated on human samples. Chouicha and Marks (2006) compared five different EIA with a cytotoxicity test on dog stool samples and found that the sensitivity and specificity were inadequate for toxin detection in dog samples. A mismatch between the results of culture or PCR and EIA was also found in a *C. difficile* infected horse (Magdesian et al., 2002; Magdesian et al., 2006; Arroyo et al., 2007; Ruby et al., 2009). Meanwhile, Medina-Torres et al. (2010) reported congruent results between EIA and cytotoxicity test in 93% of diarrhoeic horses. Compared to the culture, EIA tests were also unsuitable for toxin detection in calf faeces (Rodriguez-Palacious et al., 2006; Pirš et al., 2008). Two studies compared EIA and cytotoxicity tests in swine faeces (Post et al., 2002; Anderson and Songer, 2008). The first test had a sensitivity of 91% compared to the cytotoxicity test, but the second EIA test had a sensitivity of only 39% compared to the first EIA test. Keessen et al. (2011) compared three EIA tests and a commercial rtPCR BD GeneOhm Cdiff (BD Diagnostics, USA) on pig samples and found out that none of the compared tests is suitable as an independent method for the detection of *C. difficile* in pigs. The concordance between the results of the tests and the reference method was only in 16.9% of the samples. The best sensitivity (93%) and NPV (87.5%) were generated by rtPCR, so the authors suggested a two-stage algorithm for the detection of *C. difficile* in pigs (rtPCR as a screening test and toxigenic culture as a confirmation test of rtPCR-positive samples). In general, EIA toxin tests have a lower sensitivity in animals than in humans, and the use of EIAs for human diagnosis is not recommended without prior validation on animal samples.

Another study (Knight et al., 2014) compared five assays evaluated for use in humans on piglet faeces: GDH EIA, toxin EIA, LAMP, GeneOhm Cdiff rtPCR assay and direct culture on chromogenic agar Chrom ID *C. difficile*. None of the commercial EIA and molecular tests were suitable for use in piglets. The concordance with enrichment culture was 77.7–84.1% and the sensitivity ranged from 5.6–42.9%. Promising results were obtained with direct culture on Chrom ID agar, as 96.8% concordance with enrichment culture was observed and there was no need of prereduction, alcohol shock or additional 24h incubation.

Hopman et al. (2011) tested commercial rtPCR BD GeneOhm Cdiff for the detection of *C. difficile* in swine faeces and compared the results with the toxigenic enrichment culture: 28% of the samples were positive with toxigenic culture, while all were negative with rtPCR. So far, few rtPCR protocols have been described for veterinary use. Houser et al. (2010) published a TaqMan rtPCR assay (*tcdA*, *tcdB*, *cdtA*, *cdtB*) for the

detection of *C. difficile* in calf faeces and food. Faeces samples (n=71) were tested directly with rtPCR. According to the results, the authors suggested that rtPCR-negative samples should be cultured in a selective broth and incubated for 5–7 days. Subsequently, DNA isolated from the broths should be amplified with rtPCR to obtain more reliable results. After the enrichment step, a 7% increase of rtPCR-positive samples was seen. Avberšek et al. (2011, 2013) described two different protocols for direct detection of variant toxigenic *C. difficile* (*tcdA*, *tcdB*, *cdtB*) strains in animal faeces. The first rtPCR was based on TaqMan technology, while the second was validated on LightCycler (Roche, Germany) using two hybridisation probes. TaqMan rtPCR was also compared with two other rtPCR tests (in-house rtPCR by van den Berg et al., 2006 and PCRFast *C. difficile* A/B commercial test, ifp Institut für Produktqualitet, Germany) validated on human samples. TaqMan rtPCR was found to be the most suitable as it detected all tested variant toxinotypes and gave the best results compared to culture method. Both rtPCR tests described by Avberšek et al. (2011, 2013) were also compared to each other and to culture method. Complete concordance of both rtPCR tests was shown in 97.7% of samples, while correlation with the culture was seen in 75% of samples. As rtPCR assays for direct detection of *C. difficile* in animal faeces were not adequate as stand-alone tests due to 12% of culture positive/rtPCR negative samples, an improvement of the rtPCR was implemented. A one-day pre-enrichment step prior to rtPCR evidently increased the number of rtPCR positive samples. This procedure is also suitable as an accurate and rapid screening test for samples with a low number of *C. difficile*, as no culture positive/rtPCR negative samples were observed. It can also be used for reliable detection of *C. difficile* in subclinical animals, which could shed the bacteria and might play an important role in the spread of the infection.

rtPCR methods could also be improved by optimizing DNA extraction procedures from faeces. As *C. difficile* is sporogenic, spores are present in faeces and DNA extraction from spores could be difficult. DNA extraction from swine faeces could be improved using physical pre-treatment with repeated bead-beating or with commercial kits for DNA extraction from complex samples, which already include bead-beating in the manufacturer's instructions (Grzeskowiak et al., 2016; Avberšek et al., 2017).

Epidemiological studies often benefit from quantification of *C. difficile* in different samples. Bandelj et al. (2013) modified a protocol by Penders et al. (2005) validated for human samples. Quantitative rtPCR targeting 16S rRNA gene was validated according to MIQE guidelines for cattle faeces.

Despite promising studies on rtPCR methods in the veterinary field, cultivation of bacteria should not be neglected, as further genotyping and antimicrobial susceptibility testing cannot be performed without the isolate.

In conclusion, laboratory diagnostics of *C. difficile* is also important in veterinary medicine. Although timely detection of *C. difficile* in animals is not as critical as in human medicine, reliable, cost-effective and rapid assays are needed for diagnostics of neonatal diarrhoea, especially in piglets. Furthermore, knowledge about prevalence and genotypes of *C. difficile* in animals, food and the environment is essential to define the zoonotic potential of *C. difficile*.

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Abstract

Clostridium difficile (*C. difficile*) is an important pathogen responsible for nosocomial intestinal infections in humans. The number of community-acquired infections in patients without risk factors for infection has been increasing. In these cases, animals could act as a reservoir of *C. difficile*, which may be present in the animal digestive tract as a causative agent of enterocolitis and diarrhoea or as commensal bacteria. The overlap of human and animal *C. difficile* genotypes indicates the zoonotic potential of the bacterium, and specific genotypes capable of causing severe infections in animals and humans have been reported. Different methods are used in human medicine for laboratory detection of *C. difficile*, and a variety of commercial tests are available. Diagnostic methods are based on cultivation of *C. difficile* on selective media, detection of *C. difficile* toxins and molecular tests. All these methods could be potentially used in veterinary medicine; however, with the exception of culture methods, the use of commercial tests is limited for testing animal samples, as the test results often poorly correlate with culture results or are not capable of detecting the variant *C. difficile* toxinotypes that are common in animals. Currently, no testing algorithm is available for the detection of *C. difficile* in animals; the gold standard is toxigenic culture. Pre-enrichment in selective broth is the method of choice, though recently comparable results were obtained with direct culture on chromogenic agar Chrom ID *C. difficile.* Enzyme immunoassay (EIA) toxin tests have a lower sensitivity for animal samples, therefore the use of EIAs for human diagnosis is not recommended without prior validation on animal samples. As in human medicine, in-house molecular methods are useful for rapid detection of *C. difficile*. Real-time PCR (rtPCR) assays are not adequate as a stand-alone test; however, implementation of a one-day pre-enrichment step prior to rtPCR evidently increased method sensitivity. The sensitivity of rtPCR assays could also be improved with modified DNA extraction procedures, including repeated bead-beating.

Key words: *Clostridium difficile*, *laboratory diagnostics*, *animals*

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Laboratorijsko utvrđivanje *Clostridium difficile* u životinja: pregledni članak

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Bakterija *Clostridium difficile* (*C. difficile*) je važan patogen odgovoran za nozokomijalne crijevne infekcije ljudi. U zadnje vrijeme povećava se broj domicilnih infekcija u bolesnika bez rizičnih faktora za infekciju. U tim slučajevima životinje mogu biti rezervoar *C. difficile*, koja može biti prisutna u probavnom traktu životinje kao uzročnik enterokolitisa i proljeva ili kao komenzalna bakterija. Preklapanje ljudskih i životinjskih genotipova *C. difficile* ukazuje na zoonotski potencijal bakterije, a prijavljeni su i specifični genotipovi sposobni prouzročiti teže infekcije životinja i ljudi. U humanoj medicini primjenjuju se različite metode za laboratorijsko utvrđivanje *C. difficile*, a dostupan je i niz komercijalnih testova. Dijagnostičke metode temelje se na kultivaciji *C. difficile* na selektivnim medijima, utvrđivanju *C. difficile* toksina i molekularnim testovima. Sve se te metode mogu potencijalno primjenjivati u veterini; ali, izuzevši metode kulture, primjena komercijalnih testova ograničena je na testiranje životinjskih uzoraka, budući da su rezultati testova često u slaboj korelaciji s rezultatima kulture ili nemaju sposobnost uvtvrđivanja toksinotipova varijanti *C. difficile*, uobičajenih u životinja. U ovom trenutku nema dostupnog algoritma testiranja za utvrđivanje *C. difficile* u životinja. Zlatni standard je toksigena kultura. Prethodno obogaćivanje u selektivnom bujonu je metoda izbora, ali nedavno su dobiveni usporedivi rezultati direktnom kulturom na kromogenom agaru Chrom ID *C. difficile.* Enzimske imunopretrage (EIA) toksina imaju nižu razinu osjetljivosti za životinjske uzorke pa se stoga uporaba EIA testova ne preporučuje za dijagnostiku u ljudi, osim uz prethodnu validaciju na životinjskim uzorcima. Kao i u humanoj medicini, za brzo utvrđivanje *C. difficile* korisne su interne molekularne metode. Lančana reakcija polimerazom u stvarnom vremenu (rtPCR) nije primjerena kao samostalna pretraga, ali implementacijom jednodnevnog prethodnog obogaćivanja prije rtPCR pretrage naočigled je povećana osjetljivost metode. Osjetljivost rtPCR pretrage mogla se poboljšati i modificiranim postupcima ekstrakcije DNK, među kojima je i *bead-beating*.

Ključne riječi: *Clostridium difficile*, *laboratorijska dijagnostika*, *životinje*