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Papers should not have been previously published or be currently under consideration for publication.

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Named authors must fit the following three criteria:

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2. Drafting the article or revising it critically for important intellectual content; and
3. Final approval of the version to be published.

All people who meet the three criteria should be named as authors. Those who participate in the study but do not meet the requirements of authorship should be acknowledged as contributors.

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The text should contain introduction, material and methods, results, discussion and references. No particular format is required for review articles.

ACKNOWLEDGEMENTS

Individuals who supplied facilities, strains or reagents, or gave advice may be acknowledged. Also, supporting grants may be mentioned.

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CUTANEOUS MANIFESTATIONS IN *BLASTOCYSTIS* SPP. INFECTION

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ABSTRACT

The pathogenic potential of *Blastocystis* spp. is extremely controversial. Recently, many researchers have discussed its inductive role in the etiology of various dermatological syndromes such as palmoplantar pruritus, acute, and chronic urticaria. The growing number of urticaria patients showing improvement after eradication of *Blastocystis* spp .infection, has proven its causative nature. Herein, we present a broad overview of the modern concept of the precise parasitological verification in the routine work-up of urticaria patients.

KEYWORDS:
Blastocystis spp., urticarial, gastrointestinal symptoms

EPIDEMIOLOGY

Blastocystis spp. is one of the most common parasites in the human intestinal tract. The reported prevalence in healthy asymptomatic adults ranges from 30-50% in developing countries to 1.5-10% in industrialised nations (1).

MICROBIOLOGICAL PROFILE

Alexieff et al. first described the genus Blastocyst as a distinct organism in 1911 (2). One year later, Brumpt et al. proposed the term "*Blastocystis hominis"*, which has been widely introduced in the medical literaturethereafter (3).

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Initially regarded as a commensal organism in the human gastrointestinal tract, *Blastocystis* spp. is an anaerobic protozoan, which is now considered by many authors as a potential pathogen that causes intestinal and allergic diseases. It exists in 4 different forms (cystic, vacuolar, granular and ameboid) with size varying from 5 to 40 μm. Vacuolar and cystic forms are the most frequently found in stool samples. *Blastocystis* spp. resides in the colon and cecum.

CLINICAL MANIFESTATIONS

Clinical manifestations of *Blastocystis* infection include diarrhoea, abdominal pain, fatigue, nausea, flatulence, blood and excessive mucus in stool samples (4-6). During the past few years, *Blastocystis hominis* infection has been highly suspected as a triggering factor in the pathogenesis of acute and chronic urticaria, angioedema, palmoplantar pruritus (7) and dermatitis (8).

Despite the wide distribution of urticaria and the typical clinical presentation (transient oedematous pink or red wheals of variable size and shape that are pruritic), its etiopathogenesis is often obscured. Different foods and food additives, drugs (antibiotics, nonsteroidal anti-inflammatory drugs - NSAIDs, hormones and others), insect bites, viral, bacterial or parasitic infections, IgE-mediated type I allergic reactions, contact with allergens, physical stimuli and systemic disorders are commonly implicated in causing acute and chronic urticaria (9, 10). A large number of helminthic parasites including *Ascaris, Strongyloides, Filaria, Echinococcus, Schistosoma* and *Trichinella* have also been associated with allergic cutaneous symptoms (11).

Recent studies have suggested a high prevalence of *Toxocara canis*, *Giardia lamblia, Fasciola hepatica* and *Blastocystishominis* infection in patients with urticaria. Kantardjiev et al. analysed a series of 6 patients with urticaria, determined to be infected with amoeboid *Blastocystis* spp. and completely cured upon etiological therapy (12).

In one study (13) data of 80 patients with confirmed positive *Blastocystis* spp. infections were assessed retrospectively, revealing that 73.75% had gastrointestinal symptoms such as abdominal pain, blood and mucus in stool samples, meteorism, weight loss, perianal itching

and vomiting. 11.25% of the patients presented with skin symptoms – urticarial and dermatitis-like lesions, which resolved after specific antiprotozoal treatment. Elevated C-reactive protein and leukocytosis were observed in all patients with skin manifestations, however, no peripheral eosinophilia was identified. The authors concluded that eosinophilia is not an obligatory laboratory finding in *Blastocystis* spp. infection, with or without skin manifestations (13).

PATHOGENESIS

The pathogenic mechanism of *Blastocystis* spp.-associated urticaria remains to be determined. A variety of immuno-reactive cells populate the gastrointestinal tract, among which T- and B lymphocytes, dendritic cells, granulocytes and tissue macrophages take the main role. Chronic protozoan inoculation can serve as a constant recruitment stimulus for accumulation of other immunocompetent cells. The newly-formed functional network of neutrophils, eosinophils and lymphocytes enhance the release of histamine through cell degranulation. *Blastocystis* spp. could also activate the complement pathway with the release of C3a and C5a anaphylotoxins, which interact with specific receptors on mast cells and basophils, causing histamine release and related skin problems (14).

Some authors suggested that a co-factor induction upon *Blastocystis* infection, for example a concomitant intake of NSAIDs, might trigger mast cell degradation and profound anaphylactic reaction (15). Other hypothesis suggests that Th-2 immune response activation is needed to mediate an increase in IgE antibody synthesis (16, 17).

Recently, it was proven that only the amoeboid form of *Blastocystis* spp.expresses pathogenic potential by adhering to the gut epithelial cells lining to enhance inflammatory cells recruitment (12, 16). More than 95% of urticarial patientsshowed amoeboid form of the microorganism, which undoubtedly confirm their greater virulence (12, 18).

CONCLUSIONS

The etiological role of *Blastocystis* spp. in acute and chronic urticaria has long been controversial, but in the recent years it has been far more widely accepted. A growing number of papers emphasise the importance of performing stool microscopy and culture in patients with urticaria of unknown etiology and minor gastrointestinal symptoms when other common causative factors have been ruled out. Largepopulation studies might provide detailed evidence of the amoeboid *Blastocystis* spp.pathogenic potential as a causative factor inurticaria, thus opening new therapeutic horizons to anti-parasite medications.

REFERENCES

1. Sohail MR, Fisher PR.*Blastocystis hominis and travelers*. Travel Med Infect Dis.2005; 3:33-38.
2. Alexeieff A. *Sur la nature des formations dites "kystesdeTrichomonasintestinalis"*. Cr SocBiol. 1911; 71:296-298.
3. Brumpt E. Colite a Tetramitiusmesnili (Wenyon 1910) etcolite a TrichomonasintestinalisLeuchart 1979. Blastocystishominis n. spetformesvoisines. Bull SocPatholExot 1912; 5:725-730.
4. Giacometti A, Cirioni O, Fiorentini A. *Irritable bowelsyndrome in patients with Blastocystis hominis infection*.Eur J ClinMicrobiol Infect Dis. 1999; 18:436-439.
5. Telabasic S, Pikula ZP, Kapidzic M. *Blastocystis hominismay be a potential cause of intestinal disease.* ScandJInfect Dis. 1991; 23:389-390.
6. Vannatta JB, Adamson D, Mullikan K. *Blastocystishominis infection presenting as recurrent diarrhea*. AnnIntern Med. 1985; 102:495-496.
7. Kantardjiev V, Stratieva D, Broshtilova V. *Palmo-Plantar Lichen Planus Keratoderma.* J Res Notes. 2019; 2: 1010.
8. Kick G, Rueff F, Przbilla B. *Palmoplantar pruritussubsiding after Blastocystis hominis eradication.* ActaDermVenereol. 2002; 82:60.
9. Godse K, De A, Zawar V, et al. *Consensus statement for the diagnosis and treatment of urticaria: a 2017 update*. Ind J Dermatol. 2018; 63:2-15.
10. Kantardjiev V, Stoikova E, Broshtilova V. *Drug-induced Rowell syndrome in a male patient*. Script Scient Med 2018; 50 (4); 36-39.
11. Kolkhir P, Balakirski G, Merk H, et al. *Chronic spontaneous urticaria and internal parasites- a systemic review*. Allergy 2016; 71:308-322.
12. Kantardjiev V, Galev A, Broshtilova V. *Urticaria Associated with Amoeboid Forms of Blastocystis spp*. As J Resear Infect Diseas. 2019; 2:1-4
13. Bálint A, Dóczi I, Bereczki L, Gyulai R, e al. *Urticaria induced by Blastocystis spp.* Parasitol Res. 2014; 113:1585-1590.
14. Biedermann T, Hartmann K, Sing A, Przybilla B. *Hypersensitivityto non-steroidal anti-inflammatory drugs and chronicurticaria cured by treatment ofBlastocystis hominisinfection*.Br J Dermatol.2002;146:1113-1114.
15. Valsecchi R, Leghissa P, Greco V. *Cutaneous lesions inBlastocystis hominis infection.* ActaDermVenereol. 2004; 84:322-323.
16. Katsarou-Katsari A, Vassalos CM, Tzanetou K et al. *Acute urticarial associated with amoeboid forms of Blastocystis hominis sp.subtype 3.* Acta DermVenereol.2008; 88:80-81.
17. Yan Y, Su S, Lai R, Liao H, et al. *Genetic variability of Blastocystis hominis isolates in China*.Parasitol Res. 2006; 99:597-560.
18. Dina M, Abdel H,Hassanin O, Zuel-Fakkar NM. *Association of Blastocystis hominis genetic subtypes with urticaria*.Parasitol Res. 2011; 108:553-560.

A CASE OF PULMONARY ASPERGILLOSIS IN AN IMMUNOLOGICALLY INTACT 15-YEAR-OLD BOY

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ABSTRACT

Aspergillus is a fungus which could cause a number of infectious and allergic diseases especially in immunocompromised patients.

We report a case of 15-year-old boy with a small post-pneumonic cavity formation in the 3rd segment of the right lung. After conventional intravenous antibiotic treatment the X-ray changes were still persisting. The boy was in good general condition, without intoxication syndrome, with intact immune status. Physical examination was normal except for mild rare cough. The diagnosis was confirmed by imaging, serological and microbiological tests. Oral itraconazole (200 mg daily) was administered for 6 months along with monthly monitoring of the liver function. CT scan controls were performed in the 2nd, 4th and 6th month. Complete resolution of the cavity was observed in the final CT scan. No operative treatment was necessary.

Although typical for immunocompromised patients, an immunologically intact child without other underlying diseases can also develop pulmonary aspergillosis. Small lesions respond well to conservative treatment without surgery.

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KEYWORDS:
pulmonary aspergillosis, non-immunocompromised children, clinical presentation, diagnostic tests, treatment

INTRODUCTION

Aspergillus is a fungus which could cause a number of infectious and allergic diseases especially in immunocompromised patients. It is widespread in the environment and is often cultured from both outdoor (*i.e.* soil, plant debris) and indoor environment, including hospitals. A wide spectrum of pulmonary involvement is described in the literature (1).

The most common cause of pulmonary diseases is *Aspergillus fumigatus*, although *Aspergillus flavus* is a more common cause of allergic rhinosinusitis, postoperative aspergillosis and fungal keratitis. *Aspergillus terreus* is responsible for invasive pulmonary aspergillosis (IPA) in some institutions and unfortunately is amphotericin B-resistant. *Aspergillus niger* could be an occasional cause of IPA or *Aspergillus* bronchitis, but is also a coloniser of the airways (2, 3).

IPA is typical for individuals with severe immunodeficiency. Patients at increased risk are those with malignancy, organ transplantation or autoimmune disease, and patients in intensive care units (4-7).

Arendrup *et al* reported two cases of IPA that occurred within one day of gardening work involving tree bark chippings (5). They described severe interstitial pneumonia or miliary type picture with possible cavity formation. The authors discussed that the clinical presentation may be confused with extrinsic allergic alveolitis (EAA) (8).

The most common and best-recognised form of pulmonary involvement caused by *Aspergillus* species is aspergilloma which usually develops in a pre-existing cavity in the lung. It is composed of fungal hyphae, leukocytes, fibrin and mucus.

The most common isolate from such lesions is *A. fumigatis*, although others (*Zygomycetes* and *Fusarium*) are also described. A lot of pulmonary diseases manifesting as cavity formation could be complicated by aspergilloma, namely tuberculosis (most common), sarcoidosis, bronchiectasis, bronchial cysts and bullae, ankylosing spondylitis, neoplasm and pulmonary infection (9, 10). In a study including 544 patients with pulmonary cavities caused by tuberculosis, 11% had radiological data of aspergilloma (11, 12). It is hypothesised that the inadequate drainage of the cavity is the cause of *Aspergillus* growth.

The diagnosis of pulmonary aspergilloma is usually based on clinical picture and is confirmed by serological and microbiological methods (13). Chest X-ray could demonstrate a mass in a pre-existing cavity. The typical appearance of aspergilloma is an upper-lobe, mobile, intra-cavitary mass with an air crescent in the periphery.

Due to its high lung penetration, itraconazole is a viable option for conservative treatment in selected patients with aspergilloma (17). Long-term oral itraconazole therapy has been associated with radiological and clinical improvement in more than 50% of the cases. Occasionally a complete resolution of the cavity has been documented (14-16).

Surgery of the cavity is advised in patients with persisting X-ray changes and recurrent haemoptysis but may be associated with mortality rates of up to 7–23% (18-23).

CASE PRESENTATION

We present a clinical case of 15-year-old boy with a vague cavity formation in the 3rd segment of the right lung after pneumonia treatment.

The child had no family history of pulmonary diseases or serious diseases in the past except drug allergy against sultamicillin. The boy lives in Sofia, in a flat, and is an active swimmer.

The onset of the illness was in July 2018 when pneumonia was diagnosed due to cough, fever, general fatigue and X-ray changes. The boy was treated for 7 days with an intravenous antibiotic (amikacin) in a hospital and after that, for 7 more days with an oral antibiotic (levofloxacin) at home. However, due to the persistence of the X-ray morphology he was admitted to the hospital for diagnostic work-up.

The boy was hospitalised in the Multiprofile Hospital for Active Treatment of Pulmonary Diseases „St. Sofia" in August 2018 in a good general condition, without intoxication syndrome. Physical examination was unremarkable except for a mild rare cough.

Laboratory blood tests were performed: full blood count and liver enzymes – normal; cellular and humoral immunity – without disorders; HIV status – negative.

The initial chest X-ray upon admission showed two-sided hilar congestion; soft, gentle infiltrate was observed with clear borders from the surrounding parenchyma and hyper-lightening at the centre associated with a shadow in the right lung hilus.

On the next day chest computed tomography (CT) scan was performed, showing formation of a lobular cavity with wall thickness of up to 2 mm located in the upper right lobe (3rd segment), and soft tissue formation adjacent to the ventral contour of the cavity. Perifocally in the same segment were found multiple micronodular lesions. CT morphology was highly suggestive of aspergilloma in the right upper pulmonary lobe. In differential diagnosis another mycotic infection and less likely tuberculosis should also be considered (Fig. 2.)

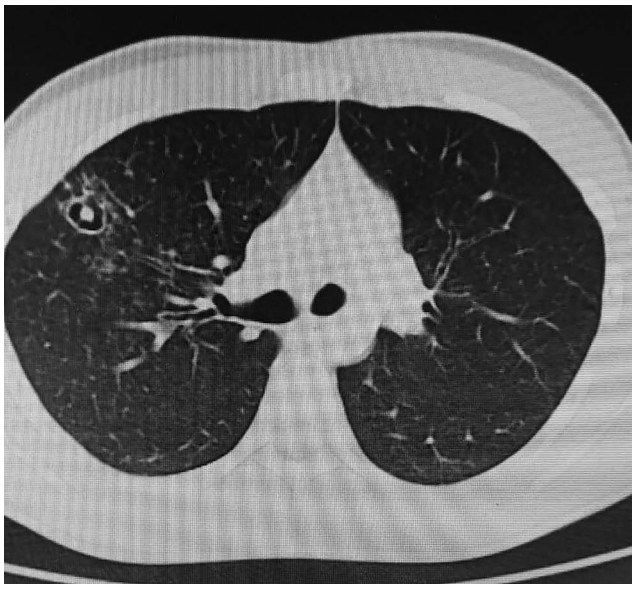

Figure 2. Initial CT findings.

Flexible fibrobronchoscopy was normal for the age, in the upper right lung there was an atypical division of the segmental bronchi. Bronchoalveolar lavage fluid (BAL) was collected for microbiology and cytology.

No bacterial growth was detected in BAL. Sputum and BAL microscopy, and culture results for tuberculosis were also negative.

Immunological tests for tuberculosis were also conducted. Tuberculin skin test (TST) was normal – 13 mm, according to Bulgarian reference values. Interferon Gamma Release Assays (IGRA) test was negative as well.

The diagnosis was confirmed with the isolation of *Aspergillus niger* from BAL submitted for fungal culture (Fig. 3). The serology test for *Aspergillus* was also positive.

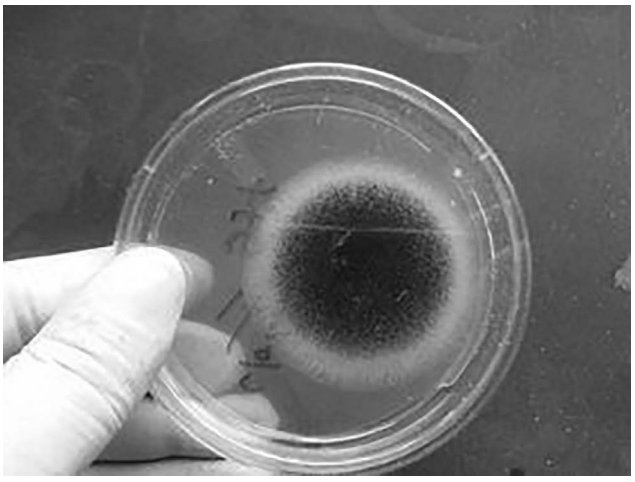

Figure 3. Culture confirmation.

Histopathology and BAL cytology showed epithelial cells, many macrophages, less neutrophils and eosinophils, spores and *Aspergillus* hyphae. The morphological diagnosis was aspergillosis (Fig. 4).

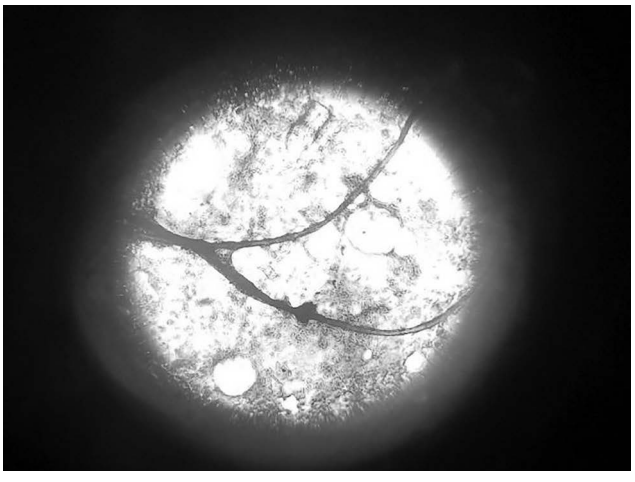

Figure 4. Microscopy confirmation.

After consultation with a paediatric thoracic surgeon an observational approach was chosen to assess the patient's condition.

In the differential diagnosis were considered also post-pneumonic cavity formation, pulmonary tuberculosis, pulmonary echinococcosis or other pulmonary parasitosis, and pulmonary mycosis.

Oral itraconazole (200 mg daily) was administered for six months along with monthly monitoring of liver enzymes. The child had no complaints during the treatment course.

CT controls were performed in the 2nd, 4th and 6th month (Fig. 5, 6 and 7, respectively). Complete resolution of the cavity was observed in the final CT. No operative treatment was necessary.

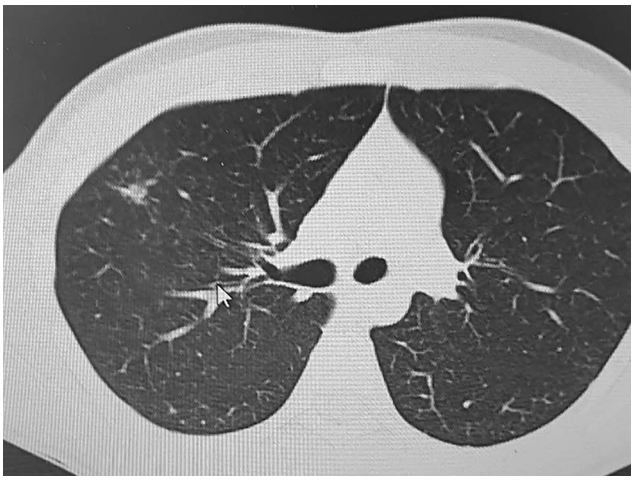

Figure 5. Control CT in the 2nd month of treatment – a small residual fibrotic focus is observed in the right upper lung at the cavity site.

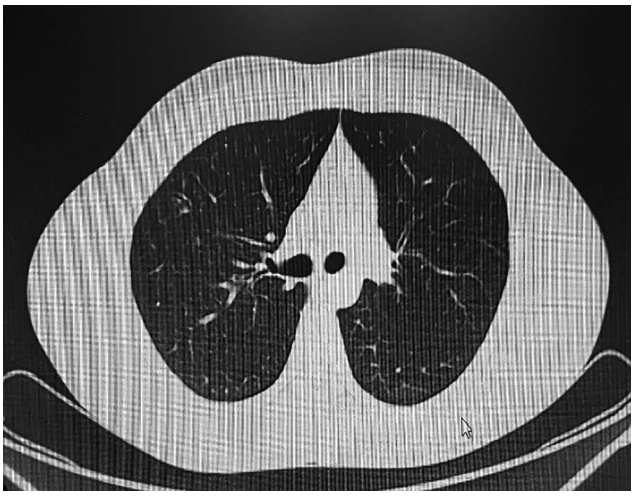

Figure 6. Control CT in the 4th month of treatment – a discrete fibrotic focus is observed in the right upper lung. There are no CT findings for focal and nodular lesions in the pulmonary parenchyma.

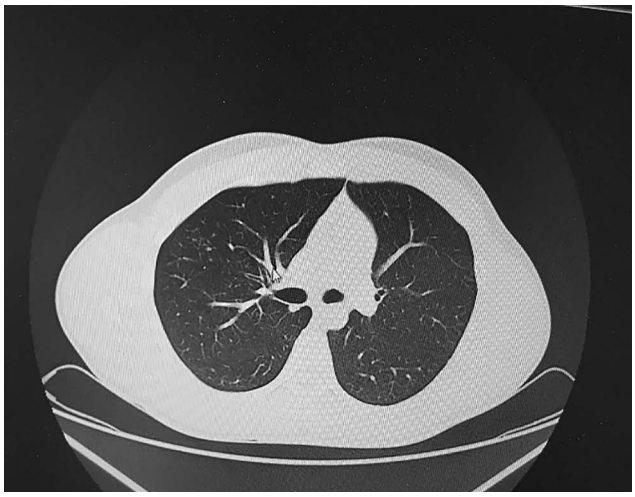

Figure 7. Control CT in the 6th month of treatment (final CT) – normal image of the lung and the mediastinal structures. There are no CT findings for focal and nodular lesions in the lung.

After the end of the antimycotic treatment *Aspergillus*-specific IgE were examined and the result was <0.1kU/l (normal <0.35).

DISCUSSION

Chronic necrotising aspergillosis is usually found in patients with chronic pulmonary disease or light immunodeficiency. Aspergilloma is usually detected in patients with pre-existing lung cavities, and allergic bronchopulmonary aspergillosis is generally observed in patients with atopy, asthma or cystic fibrosis (24, 25).

Actually, our patient did not have immune deficiency or any kind of underlying disease, and was generally a healthy teenager before the *Aspergillus* infection.

Although typical for immunocompromised patients, an immunologically intact child without other diseases can also develop pulmonary aspergillosis (24, 29). Small lesions respond well to conservative treatment without surgery.

The various clinical presentations of *Aspergillus* infection and the development of one form of the disease into another depend mainly on the immune status of the patient. There is a variety of infection hypersensitivity states in allergic aspergillosis, saprophytic infection in pre-existing pulmonary diseases and invasive forms in immunocompromised patients (26-34).

More studies are needed to better characterise the type and pathogenesis of infectious, allergic and saprophytic *Aspergillus* diseases.

REFERENCES

1. Soubani AO, Chandrasekar PH. *The clinical spectrum of pulmonary aspergillosis*. Chest. 2002; 121:1988-1999.
2. Kawamura S, Maesaki S, Tomono K, et al. *Clinical evaluation of 61 patients with pulmonary aspergilloma.* Intern Med. 2000; 39:209-212.
3. Tuncel E. *Pulmonary air meniscus sign*. Respiration 1984; 46:139-144.
4. Ratermann KL, Ereshefsky BJ, Fleishaker EL, et al. *Fulminant invasive pulmonary aspergillosis after a near-drowning accident in an immunocompetent patient*. Ann Pharmacother. 2014; 48:1225–1229.
5. Arendrup MC, O'Driscoll BR, Petersen E, et al. *Acute pulmonary aspergillosis in immunocompetent subjects after exposure to bark chippings*. Scand J Infect Dis. 2006; 38:945-949.
6. Butler L, Brockley T, Denning D, et al. *Acute Aspergillus pneumonia associated with mouldy tree bark-chippings, complicated by anti-glomerular basement membrane disease causing permanent renal failure*. Med Mycol Case Rep. 2013; 2:125-127.
7. Shimaoka Y, Ishida T, Kawasaki S, et al. *An autopsy case of Aspergillus pneumonia after inhalation of a great deal of dust in a mushroom factory*. Nihon Kokyuki Gakkai Zasshi. 2006; 44:659-664.
8. Aslam PA, Eastridge CE, Hughes FA Jr. *Aspergillosis of the lung–an eighteen-year experience.* Chest. 1971; 59:28-32.
9. Zizzo G, Castriota-Scanderbeg A, Zarrelli N, et al. *Pulmonary aspergillosis complicating ankylosing spondylitis.* Radiol Med. 1996; 91:817-818.
10. Kauffman CA. *Quandary about treatment of aspergillomas persists*. Lancet. 1996; 347:1640.
11. Rosenheim SH, Schwarz J. *Cavitary pulmonary cryptococcosis complicated by aspergilloma*. Am Rev Respir Dis. 1975; 111:549-553.
12. Sarosi GA, Silberfarb PM, Saliba NA, et al. *Aspergillomas occurring in blastomycotic cavities*. Am Rev Respir Dis. 1971; 104:581-584.
13. Meeker DP, Gephardt GN, Cordasco EM Jr., et al. *Hypersensitivity pneumonitis versus invasive pulmonary aspergillosis: two cases with unusual pathologic findings and review of the literature*. Am Rev Respir Dis. 1991; 143:431-436.
14. Campbell JH, Winter JH, Richardson MD, et al. *Treatment of pulmonary aspergilloma with itraconazole*. Thorax. 1991; 46:839-841.
15. Dupont B. *Itraconazole therapy in aspergillosis: study in 49 patients.* J Am Acad Dermatol. 1990; 23:607-614.
16. Impens N, De Greve J, De Beule K, et al. *Oral treatment with itraconazole of aspergilloma in cavitary lung cancer*. Eur Respir J. 1990; 3:837-839.
17. Tsubura E. *Multicenter clinical trial of itraconazole in the treatment of pulmonary aspergilloma*. Pulmonary aspergilloma study group. Kekkaku. 1997; 72:557-564.
18. Soltanzadeh H, Wychulis AR, Sadr F, et al. *Surgical treatment of pulmonary aspergilloma*. Ann Surg. 1977; 186:13-16.
19. Massard G, Roeslin N, Wihlm JM, et al. *Pleuropulmonary aspergilloma: clinical spectrum and results of surgical treatment*. Ann Thorac Surg. 1992; 54:1159-1164.
20. Kilman JW, Ahn C, Andrews NC, et al. *Surgery for pulmonary aspergillosis*. J Thorac Cardiovasc Surg. 1969; 57:642-647.
21. Chen JC, Chang YL, Luh SP, et al. *Surgical treatment for pulmonary aspergilloma: a 28 year experience.* Thorax. 1997; 52:810-813.
22. Garvey J, Crastnopol P, Weisz D, et al. *The surgical treatment of pulmonary aspergillomas*. J Thorac Cardiovasc Surg. 1977; 74:542-547.
23. Daly RC, Pairolero PC, Piehler JM, et al. *Pulmonary aspergilloma. Results of surgical treatment*. J Thorac Cardiovasc Surg. 1986; 92:981-988.
24. McDowell MD, Estrellado-Cruz WL. *Chronic Cavitary Pulmonary Aspergillosus with Aspergilloma in a Previously Healthy Teenager*. Am J Respir Crit Care Med. 2018;197.
25. Meng, Chen BS; Zhang, Zhongxiao BS; Liu, Xia MS; Yan, Xiuli BS; Shi, Chong BS; Liu, Na MS; Wang, Xinxin Coll; Ma, Jing MS. *Diagnosis of*

pulmonary aspergillosis-related broncholithiasis in a child undergoing bronchoscopy. Medicine. 2019 - Volume 98.

26. Munoz P, Vena A, Ceron I, et al. *Invasive pulmonary aspergillosis in heart transplant recipients: Two radiologic patterns with a different prognosis*. J Heart Lung Transplant. 2014; 33:1034-1040.
27. Falcone M, Massetti AP, Russo A, et al. *Invasive aspergillosis in patients with liver disease.* Med Mycol. 2011; 49:406-413.
28. Winters B, Custer J, Galvagno SM Jr., et al. *Diagnostic errors in the intensive care unit: a systematic review of autopsy studies.* BMJ Qual Saf. 2012; 21:894-902.
29. Luong ML, Chaparro C, Stephenson A, et al. *Pretransplant Aspergillus colonization of cystic fibrosis patients and the incidence of post-lung transplant invasive aspergillosis*. Transplantation. 2014; 97:351-357.
30. He H, Ding L, Sun B, et al. *Role of galactomannan determinations in bronchoalveolar lavage fluid samples from critically ill patients with chronic obstructive pulmonary disease for the diagnosis of invasive pulmonary aspergillosis: a prospective study*. Crit Care. 2012; 16:R138.
31. Ader F, Nseir S, Le Berre R, et al. *Invasive pulmonary aspergillosis in chronic obstructive pulmonary disease: an emerging fungal pathogen*. Clin Microbiol Infect. 2005; 11: 427-429.
32. Carrascosa Porras M, Herreras Martinez R, Corral Mones J, et al. *Fatal* Aspergillus *myocarditis following short-term corticosteroid therapy for chronic obstructive pulmonary disease.* Scand J Infect *Dis.* 2002*;* 34:224-227.
33. Bulpa PA, Dive AM, Garrino MG, et al. *Chronic obstructive pulmonary disease patients with invasive pulmonary aspergillosis: benefits of intensive care*? Intensive Care Med. 2001; 27:59-67.
34. Vandewoude KH, Blot SI, Depuydt P, et al. *Clinical relevance of* Aspergillus *isolation from respiratory tract samples in critically ill patients*. Crit Care. 2006; 10: R31.

INVASIVE PULMONARY ASPERGILLOSIS ASSOCIATED WITH INFLUENZA

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ABSTRACT

The aim of this review is to present the increased frequency of influenza-associated invasive pulmonary aspergillosis (IPA) cases reported from several countries. Classic risk factors or additional immunosuppression may not be observed in affected patients. Therefore, influenza-associated IPA might be diagnosed with a delay and consequently result in worse patient outcomes.

KEYWORDS:

invasive pulmonary aspergillosis (IPA), *Aspergillus*, influenza virus

Invasive pulmonary aspergillosis (IPA) can complicate viral infections though influenza predisposes to increased risk of bacterial superinfections (9, 10).

The development of IPA is associated with immunocompromised status but there are reports on aspergillosis in patients without major risk factors (10).

Cases of IPA in influenza patients have been described since 1952. After the influenza pandemic in 2009 there was an increase in the number of reports.

Influenza A virus is associated with IPA in most of the cases but there are also cases involving influenza B virus (10).

Despite inhaling large numbers of conidia (*Aspergillus* spores) every day, most people do not develop disease. Manifestations of the disease may range from allergic disorders to invasive aspergillosis depending on the immunocompetence of the human host, and mortality rates may reach up to 90% if there is no effective antifungal treatment (10).

Risk factors are summarised as follows:

- Immunocompromised status with underlying disease – viral hepatic cirrhosis, diabetes mellitus, acute myeloid leukaemia, prolonged neutropenia, chronic obstructive pulmonary diseases (COPD), lymphopenia (8, 9);
- Corticosteroid therapy – long-term administration of high-dose corticosteroids (methylprednisolone up to 3 weeks) for acute respiratory distress syndrome causes induced immunosuppression with leucopenia, tissue destruction, ischemic necrosis and angioinvasion (8, 9, 11);
- Transplant-related factors – immunosuppressive therapy and chemotherapy (cyclosporine, tacrolimus, cytarabine, etoposide and others) (8);
- Respiratory viruses – respiratory syncytial virus, influenza A/B and parainfluenza viruses, adenoviruses, cytomegalovirus (8);
- Blood transfusion, haemodialysis (8);
- Worsening of respiratory symptoms after initial improvement in the patient's condition (8);
- Age of more than 40 years (8);
- Antibiotic treatment of influenza patients;
- Others – mechanical ventilation, advanced liver disease, congestive heart failure and major infections (10, 11).

Role of viral infections in facilitating fungal pathogens:

- Impaired phagocytosis in macrophages;
- Impaired formation of nitric oxide (NO);
- Inhibition of apoptosis;
- Lymphopenia (decreased proliferation and migration of lymphocytes, T-cell defects);
- Decreased production of pro-inflammatory cytokines (8);
- Decreased counts of alveolar dendritic cells – the professional antigen-presenting cells (APC) (8, 11).

Upon inhalation of spores (conidia), factors contributing to angioinvasion followed by ischemic necrosis, dissemination and development of invasive

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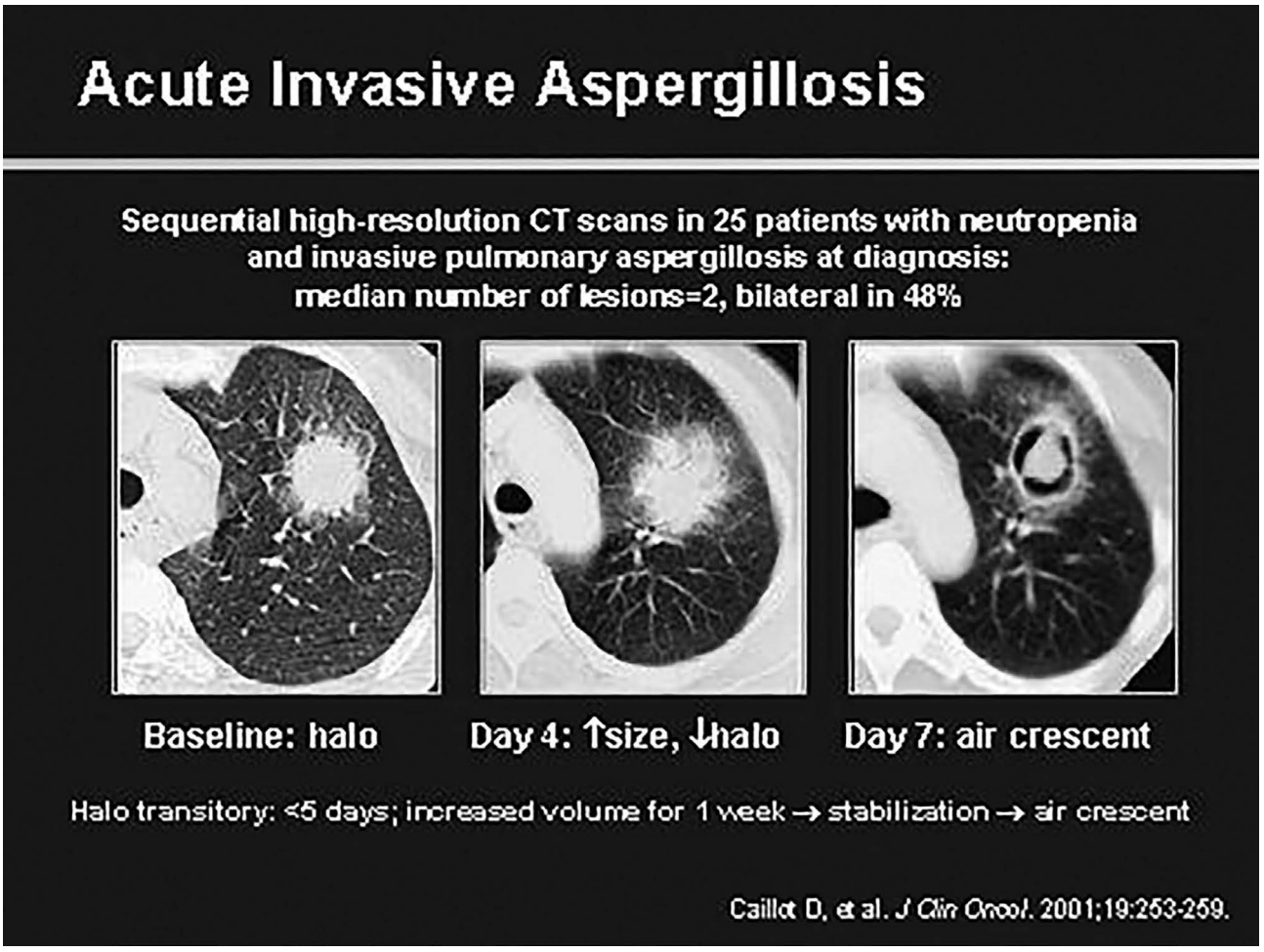

Figure 1. Radiographic presentation of *Aspergillus* pneumonia and evolution over time.
J Clin Oncol. 2001,19:253-259

aspergillosis, are impaired natural immunity, APC dysfunction (including macrophages, dendritic cells and T-lymphocytes), lack of pulmonary protection, damaged ciliary epithelium and corticosteroid-induced immunosuppression (8, 11).

The clinical forms of aspergillosis are:

- Chronic necrotizing aspergillosis with local invasion and cavitation forms (8);
- Invasive aspergillosis with angioinvasion and "halo sign" (or "air crescent sign") (8) – Fig.1.

Microbiological diagnosis can be performed with the following methods:

- Fungal culture of bronchoalveolar lavage fluid and sputum. This method has low sensitivity for the diagnosis of invasive aspergillosis, as positive results are obtained only in about 25-65% of patients. However, higher sensitivity of 63-88% was reported in patients with influenza-associated IPA (10);
- Biopsy sample collected with bronchoscopy and stained with GMS (Gomori's methenamine silver) (12);
- Serological methods for detection of galactomannan antigen, indirect immunofluorescence (IIF) for detection of antibodies in serum (1, 3);
- PCR molecular techniques for diagnosis of invasive fungal infection (4, 5, 6, 7).

The diagnosis of invasive pulmonary aspergillosis is based on a combination of clinical, microbiological and radiological criteria.

The most frequently isolated species are *Aspergillus fumigatus*, followed by *Aspergillus versicolor* and *Aspergillus niger* (14).

Specific antivirus therapy such as early treatment with oseltamivir was shown to decrease the

incidence of influenza-associated complications (11). The development of resistance to azole antifungals in *Aspergillus* strains limits treatment options in some patients and has been related to increased mortality (10). However, initiation of appropriate antifungal therapy might prove to be important in decreasing the mortality of influenza-associated aspergillosis (13).

CONCLUSION

Aspergillus species are widespread in the environment. IPA is a severe disease and immunodeficiency is the major risk factor. However, there is an increasing number of reports describing patients without the classic risk factors. Influenza patients may develop pulmonary aspergillosis as a co-infection and there are numerous reports on influenza pneumonia complicated by *Aspergillus* infection. Isavuconazole or voriconazole are used as first-line therapy (15). Patient management includes bronchoalveolar lavage, computed tomography imaging, antifungal and antiviral therapy in order to curb the co-infections.

REFERENCES

1. Kantardjiev T. *Etiologic diagnosis and etiotropic therapy of mycoses*. 2012, Sofia, National Centre of Infectious and Parasitic Diseases.
2. Kantardjiev T, Levterova V, Brankova N, Ivanov I, Angelov P, Panaiotov S. *Role of fluorescent amplified fragment length polymorphism analysis in taxonomy, identification and epidemiological examinations of yeast pathogens*. Biotechnol Biotechnol Equip. 2006; 20(1):103-106.
3. Kantardjiev T**,** Levterova V, Panaiotov S, Ivanov I. *Use of Amplified Fragment Length Polymorfhism analysis as a tool for identification and typing of yeast isolates*. Mykologia Lekarska (Medical Mycology). 2004; 11(2):113-117.
4. Buess M, Cathomas G, Halter J. *Aspergillus-PCR in bronchoalveolar lavage for detection of invasive pulmonary aspergillosis in immunocompromised patients***.** BMC Infect Dis. 2012; 12(1):237.
5. Johnson GL, Bibby DF, Wong S, Agrawal SG. *A MIQE-compliant real-time PCR assay for Aspergillus detection*. PLoS One. 2012; 7(7):e40022.
6. Valero C, Cruz-Villar L. *New Panfungal Real Time PCR Assay for diagnosis of Invasive Fungal Infection*. J Clin Microbiol. 2016; 54:2910-2918.
7. Somogyvari F, Horvath A, Serly J, Majoros H, Vagvolgyi C, Peto Z. *Detection of Invasive Fungal Pathogens by Real Time PCR and High-resolution Melting Analysis*. 2012; 26(6):979-983.
8. Garcia-Vidal C. *The interaction between Influenza and Aspergillus*. ECCMID April 2018, Infectious Diseases Department.
9. Fischer J, Walker D. *Invasive pulmonary Aspergillosis associated with influenza.* JAMA. 1979; 241(14):1493-1494.
10. ECDC. *Influenza-associated invasive pulmonary aspergillosis*, Europe. Report from 30 November 2018, Stockholm.
11. Lat A, Bhadelia N, Miko B. *Invasive Aspergillosis after pandemic (H1N1) 2009.* Emerg Infect Dis. 2010; 16(6):971-973.
12. Shah MM, Hsiao EI, Kirsch CM, Gohil A, Narasimhan S, Stevens DA. *Invasive pulmonary aspergillosis and influenza co-infection in immunocompetent hosts: case reports and review of the literature.* Diagn Microbiol Infect Dis. 2018; 91(2):147-152.
13. van de Veerdonk FL, Kolwijck E, Lestrade PP, Hodiamont CJ, Rijnders BJ, van Paassen J, et al. *Influenza-asociated Aspergillosis in critically ill patients*. Am J Respir Crit Care Med. 2017; 196(4):524-527.
14. Nancy F. Crum-Cianflone. *Invasive Aspergillosis Associated With Severe Influenza Infections*. Open Forum Infect Dis. 2016; 3(3):ofw171.
15. Koehler P, Bassetti M, et al. *Intensive care management of influenza-associated pulmonary aspergillosis.* Clin Microbiol Infect. 2019 [Epub ahead of print].

SEROPREVALENCE OF WEST NILE VIRUS IN BULGARIA, 2018

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ABSTRACT

In Bulgaria, the first laboratory-confirmed West Nile neuroinvasive disease (WNND) case occurred in 2015 and more cases have been reported ever since. The aim of our study was to track the current circulation of West Nile virus (WNV) in the country. We collected 1830 serum samples from healthy people of every district in Bulgaria. Commercially available ELISA kits were used to detect specific anti-WNV IgG antibodies. All positive samples were tested for specific IgM antibodies using the same method. Possible risk factors were identified by calculating odds ratio. Specific IgG antibodies were detected in 22 of the samples (1.2%, CI 0.8% to 1.8%). No IgM antibodies were detected in the positive samples. The highest seroprevalence rates were found in the districts of Pleven, Varna, Silistra and Yambol. This study showed the continuous circulation and spread of WNV in Bulgaria.

KEYWORDS:

West Nile virus, seroprevalence, antibodies, neuroinvasive disease

INTRODUCTION

West Nile virus (WNV) is a mosquito-borne flavivirus emerging in Europe and America. As many other flaviviruses, WNV is maintained in nature in a cycle which involves mosquitoes and vertebrate hosts. WNV uses *Culex* mosquitoes as vectors, and domestic and wild birds as amplifying hosts. Humans and horses are an incidental end host of the virus.

Most human infections with WNV are asymptomatic (~80%). About 20% of WNV infections result in influenza-like fever, called West Nile fever, and less than 1% cause neuroinvasive diseases (West Nile neurologic disease, WNND). Clinicians mostly recognise neuroinvasive infections which represent less than 1% of WNV infections, but the actual number of infected people is a lot higher.

In Europe, WNV emerged in Romania in 1997 when 352 WNND cases were confirmed (6). In the following years, a number of WNV outbreaks occurred in Europe, including in the Balkans: 609 cases in Greece (2010-2013), 371 cases in Serbia (2012-2013) (5) and 47 cases in Romania (7).

In Bulgaria, the first laboratory-confirmed WNND case occurred in 2015 and more cases have been reported ever since. Following the first confirmed case, a nationwide seroprevalence study was conducted in 2015 (1). An overall seroprevalence of 1.5% was found, varying from 0 to 10% among districts. The aim of our study was to track the current circulation of WNV in Bulgaria.

MATERIAL AND METHODS

Between February and March 2018, a total of 1830 serum samples were obtained from healthy people (57.7% females and 42.3% males) from each of the 28 districts of Bulgaria. The age of the people ranged between 15 and 93 years, with a median age of 57. The samples were transported and stored at -20°C before testing.

Commercially available ELISA kits were used for detection of specific anti-WNV IgG antibodies (Euroimmun, Germany), following the manufacturer's instructions. Samples resulting positive were further tested for the presence of specific IgM antibodies using ELISA kits from the same manufacturer.

In order to identify possible risk factors, univariate analysis was performed calculating the odds ratio (OR) and 95% confidence interval (CI).

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Table 1. WNV seroprevalence rates by district (Bulgaria 2018).

District	Positive (no)	Tested (no)	Prevalence rate	lower CI	upper CI
Blagoevgrad	1	60	1.67%	0.29%	8.86%
Burgas	2	81	2.47%	0.68%	8.56%
Dobrich	0	70	0.00%	0.00%	0.00%
Gabrovo	1	60	1.67%	0.29%	8.86%
Haskovo	1	70	1.43%	0.25%	7.66%
Kardzhali	0	60	0.00%	0.00%	0.00%
Kustendil	0	60	0.00%	0.00%	0.00%
Lovech	0	60	0.00%	0.00%	0.00%
Montana	2	80	2.50%	2.50%	8.66%
Pazardzhik	0	60	0.00%	0.00%	0.00%
Pernik	0	60	0.00%	0.00%	0.00%
Pleven	3	70	4.29%	1.47%	11.86%
Plovdiv	1	70	1.43%	0.25%	7.66%
Razgrad	0	60	0.00%	0.00%	0.00%
Ruse	1	70	1.43%	0.25%	7.66%
Shumen	0	60	0.00%	0.00%	0.00%
Silistra	2	70	2.86%	0.79%	9.83%
Sliven	0	60	0.00%	0.00%	0.00%
Smolyan	0	60	0.00%	0.00%	0.00%
Sofia city	1	80	1.25%	0.22%	6.75%
Sofia district	1	80	1.25%	0.22%	6.75%
Stara Zagora	1	60	1.67%	0.29%	8.86%
Targovishte	0	60	0.00%	0.00%	0.00%
V Tarnovo	0	60	0.00%	0.00%	0.00%
Varna	2	60	3.33%	0.92%	11.36%
Vidin	0	49	0.00%	0.00%	0.00%
Vratsa	1	70	1.43%	0.25%	7.66%
Yambol	2	70	2.86%	0.79%	9.83%
TOTAL	22	1830	1.20%	0.80%	1.81%

RESULTS

Specific WNV IgG antibodies were detected in 22 of the 1830 samples (Table 1) (1.2%, CI 0.8% to 1.8%). No anti-WNV IgM antibodies were detected in the positive samples. The districts with the highest seroprevalence rates were Pleven (4.29%, CI 1.47% to 11.86%), Varna (3.33%, CI 0.92% to 11.36%), Silistra (2.86%, CI 0.79% to 9.83%) and Yambol (2.86%, CI 0.79% to 9.83%).

There were no significant differences between the positive female (1.42%) and male (0.9%) groups: OR 1.58 (CI 0.64 to 3.9, p value=0.32). Age (40 and above) also did not show any influence on WNV infection (OR 1.54, CI 0.62 to 3.81, p value=0.35).

DISCUSSION

The current WNV seroprevalence rate of 1.2% is consistent with the previous study of ours, which showed an overall rate of 1.5%. Interestingly, there are differences regarding the spread of the viral circulation between the current and the previous nationwide seroprevalence study. Three years ago, the highest seroprevalence rates were found in the districts of Sofia province and Vidin (1). In the current study the prevalence rates for these districts were 1.25% and 0%, respectively, which might be showing better mosquito management and control. On the other hand, the highest seroprevalence rates were in the districts of Pleven, Varna, Silistra and Yambol, which is consistent with the previous results, demonstrating high WNV rates in the districts of Pleven, Silistra and Yambol (2%, 6% and 2%, respectively). This is also expected, since Pleven and Silistra are bordering the river of Danube with favourable conditions for mosquito populations; Varna is bordering the Black Sea, as well as a couple of lakes (Lake Varna and Lake Beloslav); and Yambol is bordering the Tundzha river and is close to the neighbouring countries of Greece and Turkey, where there were numerous WNV cases in the recent years (3,4). We found high seroprevalence rate in the district of Burgas as well – 2.47% (CI 0.68% to 8.56%), where a total of 4 WNND cases were detected in 2018, representing almost 1/3 of the reported WNND cases that year (2).

The present country-wide WNV seroprevalence study shows that the virus continues to circulate and spread to new foci. Moreover, in 2018 there were an unexpectedly high number of WNND cases in Bulgaria. This suggests improved disease recognition by clinicians, but nevertheless further studies should be performed examining the circulation of the virus in reservoir hosts and vectors as well.

REFERENCES

1. Christova I, Panayotova E, Tchakarova S, Taseva E, Trifonova I, Gladnishka T. *A nationwide seroprevalence screening for West Nile virus and Tick-borne encephalitis virus in the population of Bulgaria*. J Med Virol. 2017; 89(10):1875-1878.
2. Christova I, Panayotova E, Trifonova I, Taseva E, Gladnishka T. *Unexpectedly high number of West Nile neuroinvasive diseases in Bulgaria in 2018*. Probl Infect Parasit Dis. 2018; 46(2):16-19.
3. Ergunay K, Bakonyi T, Nowotny N, Ozkul A. *Close relationship between West Nile virus from Turkey and lineage 1 strain from Central African Republic*. Emerg Infect Dis. 2015; 21(2):352-355.
4. Lustig Y, Sofer D, Bucris ED, Mendelson E. *Surveillance and Diagnosis of West Nile Virus in the Face of Flavivirus Cross-Reactivity*. Front Microbiol. 2018; 9:2421.
5. Petrović T, Šekler M, Petrić D, Lazić S, Debeljak Z, Vidanović D, et al. *Methodology and results of integrated WNV surveillance programmes in Serbia*. PLoS ONE. 2018; 13(4):e0195439.
6. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. *West Nile encephalitis epidemic in southeastern Romania*. Lancet. 1998; 352(9130):767-771.
7. Popescu CP, Florescu SA, Badescu D, Ceianu CS, Zaharia M, Tardei G, Codreanu D, Ceausu E, Ruta SM. Re-emergence of severe West Nile virus neuroinvasive disease in humans in Romania, 2012 to 2017 – implications for travel medicine. Travel Med Infect Dis. 2018; 22:30-35.

IMPACT OF THE ENVIRONMENT ON DEVELOPMENT OF HELMINTH AND PROTOZOAN INVASIVE ELEMENTS AND CONTEMPORARY METHODS FOR SANITARY-PARASITOLOGICAL DIAGNOSTICS: REVIEW

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ABSTRACT

According to the World Health Organisation, more than 1.5 billion of the world population is affected by parasitic diseases caused by geohelminths. The number of persons suffering from foodborne and waterborne protozoan diseases is similar. In developed countries, including Bulgaria, systematic sanitary and parasitological studies of soil and water are the basis for monitoring and control in protecting public health. Occurrence and prevalence of human parasitic infections is determined by the peculiarities of life cycle of parasites, peculiarities of hosts as reservoir sources, the abiotic and biotic factors of the environment as a complex providing conditions for the development or sterilisation of different parasite stages, as well as socioeconomic factors that play a leading role in the whole epidemiological process.

Systematic sanitary-parasitological studies require the application of classical and novel reliable, sensitive and practical diagnostic methods that are also easy to perform, economical and efficient enough.

KEYWORDS:

sanitary parasitology, factors, methods

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Sources of parasitic diseases are infected humans or animals, where the parasites produce invasive elements. The power of the source is determined by the amount of infectious material excreted per day in the external environment, and depends on the type of parasite and its reproductive capacity.

The mechanism of parasite transmission and penetration is specific and is accomplished through a number of biotic, abiotic and social factors that provide the opportunity of the invasive forms to enter the host body. Certain features of the host, e.g. resistance, immunity, age, etc., as well as those of propagative stage of the parasite, clarify the role of biological factors in the epidemic process of parasitic diseases. The mechanisms of dissemination, transmission and penetration of invasive stages in the macroorganism of the healthy and susceptible human population, under the specific economic, cultural and household conditions, are the social factors in the epidemic process. These two types of factors (biological and social) determine the course of the epidemic process (1).

In some cases the release of parasites into the environment occurs through faeces, urine, sputum, vaginal discharge (anal-oral and urogenital anthroponotic diseases). Important for their subsequent distribution is either entering the irrigation systems, drinking water sources or contamination of ground-growing vegetables, fruits, household objects, hands, food, etc. Other parasites circulate in the blood and lymph fluid of hosts or are found in their muscles, internal organs, or skin (2).

The most commonly reported routes for transmission of infection, particularly in humans, are as follows (3):

- Ingestion of eggs, cysts, larvae or hosts with invasive stages of the parasites (*Entamoeba* spp., *Giardia* spp., *Ascaris* spp., *Toxocara* spp., *Enterobius*

vermicularis, *Trichuris* spp., *Echinococcus* spp., *Blastocystis* spp., *Trichinella* spp.)
- Inoculation of invasive stages by host or vector (malaria plasmodia, leishmania, filaria).
- Direct penetration of invasive stages of parasites into the human body through the skin (*Ancylostoma* spp., *Schistosoma* spp., *Strongyloides* spp.)

ABIOTIC AND BIOTIC FACTORS INFLUENCING THE DEVELOPMENT AND SURVIVAL

Soil conditions are an important factor for the egg development rate. Main limiting factors are temperature and soil moisture. Eggs and other invasive stages of helminths have different survival time in the soil. For example, the eggs of *Ascaris lumbricoides*, *Teania saginata,* and *Trichuris trichiurus* can survive in soil for several months, while cysts of *Entamoeba histolytica* - less than 10 days.

Survival of parasites outside the host`s body depends on environmental conditions. The eggshell is one of the most resistant biological structures that offer a high degree of protection for the developing embryo. It is highly impermeable and only lipid solvents, gases, and water molecules pass through it (4).

Factors influencing the survival of cysts of parasitic protozoa and helminth eggs and larvae can be grouped into three categories:
- Physical factors - temperature, sunlight, ultraviolet radiation, etc.
- Chemical factors - oxygen concentration, acidity (pH) of the soil, various chemicals, etc.
- Biological factors - pathogenic and ovicidal fungi, protozoa, invertebrates, etc.

Temperature is one of the physical factors mainly affecting the development of invasive elements, such as size, maturation, survival and infectivity of the free parasite stages. At high temperatures, nematode larvae slow down their function, fall into heat stupor, and die above 60°C. Some geohelminths avoid these unfavourable for their development conditions by transition from one stage to another; different species can tolerate different temperature range (8). Under pasteurisation conditions (90°C), high temperature is used for the remediation of sludge from invasive eggs of *Ascaris* spp. Eggs of *Taenia saginata* lose viability in five minutes at 71°C and the eggs of *Necator americanus* in 50 minutes at 45°C (9).

On the other hand, low temperatures (8.9°C to 15.6°C) prevent the full embryonation of the egg. Under experimental conditions *Ascaris* spp. eggs are stored in a refrigerator at temperature of about 7-8°C. In some parasitic species low environmental temperatures can have a limiting effect, while in others they have no significant influence. For example, the 3rd-stage larvae of *Trichostrongylidae* species survive at temperatures below -28°C in pastures in Poland (5).

A high percentage of lost viability is observed when eggs and partially developed embryos of *Trichiuris* spp. are exposed to very low temperatures (-9°C to -12°C) (6). The larvae release is sharply reduced after prolonged exposure (from 77% to 47%). The *Ascaris suum* eggs remain viable for a period of 40 days when they are exposed to temperatures ranging from -18°C to -27°C (7).

Another important factor for the survival of parasites and their invasive elements is light and ultraviolet radiation. Both factors have a direct impact on some of the stages in the life cycle of parasitic nematodes (10). It is believed that the colouring of eggshells of certain zooparasitic nematodes is associated with additional protection in relation to ultraviolet radiation.

Eggs of *Trichiurus* spp. are significantly more resistant to the impact of light. It is supposed that the dark pigmentation of their eggshell protects them from the shorter wave lengths of ultraviolet light. For example, even very short exposure of *Ascaris* spp. eggs is sufficient for disturbances of embryonic development to occur, and longer is lethal (6).

Humidity of the soil is a major factor for the survival of helminths and many of them practically are unable to survive in the dry soil substrate. It is of great importance whether soil drought occurs slow or fast. For example, *Trichostrongilus colubriformis* can survive 164 days when drought occurs slowly within 3-4 days (11). Eggs of some zooparasitic nematodes lose their water very slowly when subjected to drying and this allows the larva to

survive water stress. However the eggs of *Ascaris* spp. survive at low and zero humidity levels only up to 3 days regardless of temperature (7). The optimal conditions for their normal development are humidity about 80% and temperature above 20°C.

EFFECTS OF DRYING ON THE VIABILITY OF PARASITE EGGS IN SLUDGE

There is a correlation between eggs viability and moisture content of the sludge. Eggs inactivation increases with decrease in moisture in the sludge from the drying layer. The lowest humidity levels at which all eggs of *Ascaris* spp. and *Toxocara* spp. become inactive are 5% in autumn, 7% in winter, 8% in spring and 15% in summer. It has been found that both temperature and decrease in moisture content play a role in the inactivation of these parasites. Drying the sludge on air to very low humidity levels leads to complete destruction of the parasites present there.

Another group of factors affecting the survival of the parasites and their elements involved in invasion are the chemical ones. The most important from these factors are soil oxygen concentration and pH.

The lack of oxygen suppresses the overall metabolism of many nematodes and affects their development and survival. For *Ascaris* spp. eggs the development rate sharply decreases under low oxygen concentration as they are obligate aerobes (12). Non-embryonated eggs can survive for several weeks at room temperature under anaerobic conditions but their growth is inhibited. In nature eggs of *Ascaris* spp. can adapt to developing in a low-oxygen environment. Adult helminths inhabiting the human intestinal tract live in practically anaerobic conditions and have primary anaerobic metabolism (13, 14). However, their eggs require mandatory oxygen exposure to continue their normal development.

ACIDITY (PH)

Parasite eggs are considered to be very resistant to extreme pH values (15). The optimal pH for the development of *Necator americanus* eggs is about 6. The ecological significance of this is that the faeces and soil provide optimal pH for hatching as well as contain the necessary nutrients and electrolytes for further development of the larvae to the invasive stage (15).

The eggs of *Ascaris* spp. can be embryonated in a wide range of relatively toxic solutions such as 14% hydrochloric acid, 9% sulfuric acid, 8% acetic acid, 0.4% nitric acid, 0.3% carboxylic acid, 0.5% sodium hydroxide, 1% mercuric chloride, 4% formaldehyde as well as in a number of chemicals used for cleaning and disinfection. Concentrations of ozone and chlorine at levels of 4.0 and 40 mg/l, respectively, were found to destroy the eggs of *Shistosoma mansonia* while ozone has no effect on the eggs of *Ascaris* spp. and *Hymenolepis* spp. (16). The resistance of these eggs to toxic substances is due mainly to the structure of the inner membrane of the eggshell, which is lipoid in nature (17).

BIOTIC FACTORS

The development of parasitic eggs and protozoan cysts also depends on different biological factors. Soil pathogenic fungi are capable of attacking and destroying eggs of *Ascaris lumbricoides* (18). Under experimental conditions this occurs for several days or weeks. The rate of the ovicidal effect depends on the type of ovicidal fungi and the active substances they release. The parasitic fungus *Cylindrocarpon radicola* penetrates and destroys the helminth eggs (19, 20). A number of invertebrates, especially insects and snails, can also mechanically kill the eggs of helminths. It has been found that between 10% and 20% of the eggs of *Ascaris* spp. are discharged from *Planorbis planorbis, P. corneus, Bithynia tentaculata*, structurally damaged and incapable of further development. From 8% to 10% of the eggs develop only to the gastrula. The embryogenesis of the remaining eggs is delayed by 10 to 15 days (21).

ASSESSMENT OF THE VIABILITY OF HELMINTH EGGS AND LARVAE

Determination of the viability and number of discovered eggs/larvae for each sample is necessary and important in order to carry out risk assessment in accordance with certain international and national standards and guidelines (22, 23).

The most widely used method of assessing the

viability is incubation. Solutions of sulfuric acid (24, 25) and formalin (26) are used to incubate the isolated eggs at incubation temperatures ranging between 22°C and 26°C and duration of the incubation period between 21 and 30 days (26). The solution of sulfuric acid (0.1N) was reported to give the best result, between 75-80% (22) and 83-92% (27) viability, followed by formalin solution (75-80%) (26). The disadvantage of incubation in determining the viability of helminth eggs is the duration of time. The morphological integrity of the eggs and their response to dye staining, as well as their characteristic features as size, shape, and presence of visible larvae are used as a criterion for the viability of the eggs.

This is avoided by the use of dyes differentiating viable from non-viable eggs based on the permeability of the eggshells. The most widely used in practice are the Lugol solution (28), Safranin O (24) and Eosin Y (29). When comparing the results using different dyes versus conventional incubation, was found that conventional incubation detected 86% of viable eggs, which was lower than the viable eggs determined by staining with safran (97%), crystal violet (92%) and methylene blue (87%). The lowest viability (39%) was reported for trypan blue staining (30). Disadvantage of the colouring is that some dyes are toxic to the embryos, so the sample testing should be done within a few minutes after the application of the dye (31).

CLASSICAL METHODS FOR DETECTION AND IDENTIFICATION

Most of the methods used for detection and grading of external environment samples for the presence of helminth eggs, larvae and protozoan cysts are performed by standard light microscopy and are called conventional methods. They include sedimentation followed by an extraction stage and then flotation prior to microscopy (32). However, all these steps may vary depending on the type of the sample, its amount, pretreatment of the sample and the type of searched parasites and their different stages that can be identified in the environment.

Samples from different sources and their quantity are distinguished in the individual test matrices – sewage, sludge, compost, soil. Detection of helminth eggs and protozoan cysts is hampered by their uneven distribution in the environment. This feature can be compensated by the collection of a large number of single samples from a given habitat and their homogenisation. This method is used more often in samples of sludge, compost, faeces (26).

In addition, eggs of *Ascaris* spp., *Trichuris* spp., and *Toxocara* spp. are highly resistant to degradation of sediments and the removal or even the inactivation of the parasites requires subsequent disinfection steps. However, some of them maintain their invasive potential even after severe treatment conditions. For example, Maya et al. (24) assessed the degree of inactivation of *Ascaris lumbricoides, Ascaris suum, Toxocara canis,* and *Trichuris trichiura* eggs when subjected to 80°C and pH 12.1 and found that less than 25% of these parasites were inactivated under these conditions.

An important step is the concentration of samples and the separation of helminth eggs and protozoan cysts from sludge containing multiple compacted particles. This is often done by sedimentation, which may be passive, or by using a different speed centrifugation. So far, however, there is no commonly accepted methodology by the different laboratories in the world (33).

A critical step during separation of the eggs from the rest of the particles is flotation. Different solutions such as $MgSO_4$, $ZnSO_4$, NaCl, $NaNO_3$ and sucrose solutions are used, but a saturated solution of NaCl is the most widely used for flotation.

CURRENT METHODS OF DIAGNOSIS

PCR techniques are emerging as very specific, sensitive and rapid methods for detection of different pathogens in different matrices of waste water to soil and food products (34, 35). Among the PCR methods developed for detecting pathogens are the quantitative polymerase chain reaction (qPCR), multiplex polymerase chain reaction (mPCR) and drip-digital polymerase chain reaction (ddPCR) (36). One of the main obstacles to the efficient detection of eggs by using molecular methods is the extraction of a nucleic acid with good quality

and quantity, which is hampered by their hard shell (37). Furthermore, the presence of large amounts of suspended solids in the samples also hinder the extraction of nucleic acid (38) and can inhibit the PCR reactions. Separation of eggs from these solid particles is carried out prior to extraction of DNA (38) using flotation and/or sedimentation steps. The ability to analyse species-specific gene sequences by PCR makes the method preferred in cases of identification difficulties with standard morphological methods (39). Several other studies have shown that qPCR is much more sensitive than conventional microscopic methods for detecting helminths in different environmental samples (40).

In Bulgaria sanitary-parasitological surveys are carried out by the Regional Health Inspectorates (RHI). Environmental investigations for the presence of parasites and their invasive elements are conducted annually and results are announced in the annual reports. For the period 1995-2009 were examined 6414 samples of soil from settlement foci of soil-transmitted helminthiasis, sandboxes in childcare facilities and parks. In 54 samples (0.84%) were found eggs of *A. lumbricoides*, *T. trichiuris* and larvae of *Trichostrongylidae* spp. (41). The relative share of positive samples shows a sustainability trend from 0.52% (1996) to 0.54% (2008). Several other studies have been published in recent years by Georgieva et al, 1999 and 2005 (42, 43), Muhtarov, 2016 (44) and Popova, 2018 (45). In samples from playgrounds, gardens, and garbage bins in Stara Zagora, the authors found *Toxocara* spp. eggs in 17 samples, *Trichiurus* spp. eggs in 5 samples, *Taenia* spp. eggs in 4 samples, and *Dipilydium caninum* in 3 samples (42, 43). A total of 301 samples from environment were investigated in Kardzhali district and in 71 (23.59%) were found parasitic invasive forms, from which about 1% were eggs of *Taenia* spp. (44). Similar results were obtained in a sanitary-parasitological study conducted in Plovdiv region, where about 2% of the samples were contaminated with eggs of *Taenia* spp. (45).

For the period from August to October 2017, in the National Reference Laboratory for Diagnosis of Parasitic Diseases at the NCIPD were tested 180 samples of soil, sand, open water sources and sludge from wastewater treatment plants. Conventional (microscopic) and biomolecular (PCR) diagnostic methods were used. In the studied samples of different substrates with microscopic analysis were found 16 (9%) positive: 5 positive for nematode larvae, 2 positive for eggs of *Ancylostoma* spp. and 9 positive specimens for oocysts of *Cryptosporidium* spp. By using PCR methods, the number of positive samples increased to 22 (11%): 4 positive for oocysts of *Toxoplasma gondii*, 1 positive for oocysts of *Cryptosporidium* spp., 1 positive for *Toxocara canis* eggs (46). In this respect, our data on sensitivity of PCR techniques is similar to data found in the literature. Furthermore, biomolecular methods allow species identification with high accuracy including specimens that are unfit for morphological analysis or are morphologically identical (e.g. *Taenia* spp. eggs).

In conclusion, we can state that in order to perform quality sanitary-parasitological studies it is necessary to include the biomolecular methods for species identification of the detected helminth eggs and protozoan cysts. This would significantly improve the quality of the research on environmental samples for the presence of invasive parasitic elements as well as the measures for surveillance and control of parasitic diseases.

REFERENCES

1. Anderson RM, May RM. *Regulation and stability of host-parasite population interactions. 1. Regulatory processes*. J Animal Ecol. 1978; 47(1):219-247.
2. Cheng, TS, (1986). General Parasitology. Academic Press, Inc. Orlando, Florida, ISBN: 0-12-170755-5, 827 pp.
3. Chappell, LH, (1993). *Physiology and nutrition*. Modern Parasitology: A Textbook of Parasitology, Second Edition, 157-192.
4. Feachem, RG, Bradley, DJ, Garelick, H. and Mara, DD, (1983). *Sanitation and Disease: Health Aspects of Excreta and Wastewater Management.* John Wiley and Sons, Chichester, UK.
5. Wharton DA. *Ascaris sp.: Water loss during desiccation of embryonating eggs*. Exp Parasitol. 1979; 48:398-406.
6. Nolf LO. *Experimental Studies on Certain Factors influencing the Development and Viability of the Ova of the Human Trichuris as compared with those of the Human Ascaris*. Am J Hyg. 1932; 16(1):288-322.
7. Cram EB. *The Influence of Low Temperatures and of Disin-fectants on the Eggs of Ascaris lumbricoides*. J Agric Res. 1924; 27(3):167-175.
8. Parsell DA Lindquist S. (1994) *Heat shock proteins and stress tolerance. In: The biology of heat Shock Proteins and Molecular Chaperones*, edited by RIMorimoto, A.Tissieres and C.Georgopoulos: 457-495. Cold Spring Harbor Lab Press.
9. Barrett J. *Studies on the induction of permeability in Ascaris lumbricoides eggs*. Parasitol. 1976; 73:109-121.
10. Hollaender A, Jones MF, Jacobs L. *The effects of monochromatic ultraviolet radiation on eggs of the nematode, Enterobius vermicularis. I. Quantitative response.* J Parasitol. 1940; 26:421-432.

11. Allan GS, Wharton DA. *Anhydrobiosis in the infective juveniles of Trichostrongylus columbriformis (Nematoda Trichostrongylidae).* Int J Parasitol. 1990; 20:183-192.
12. Atkinson HJ. (1980) *Respiration in nemathods. In Nematodes as Biological Models*, Vol.2, ed by BM Zuckerman, pp 101-142, New York & London Academic Press.
13. Hass DK, Todd AC. *Extention of a technique for hatching ascarid eggs in vitro*. Am J Vet Res. 1962; 23:169-170.
14. Anya AO. *Experimental studies on fisiology of hatching of eggs of Aspiculuris tetraptera Schulz (Oxyuroidea; Nematoda)*. Parasitol. 1966; 56:733-744.
15. Kiff RJ, Lewis-Jones R. (1984) *Factors that govern the survival of selected parasites in sewage sludges. Sew. Sludge Stabilisation Disinfect.* Ellis Horwood Ltd., Chichester, Engl. 1984.p 452-461, fig 3, 18 ref.
16. Reimers RS, Little MD, Akers TG, Henrigues WD, Badeaux R, McDonnell D, Mbela K. (1990). *Persistence of pathogens in lagoon-stored sludge.* Available from USA Env.Prot. Agency, Risk Red. Eng. Lab, Cincinnati, EPA / 600 / S2-89 / 015 Jan 1990.
17. Perry, RN (2002) *Hatching. In The biology of Nematoses* Ed.DLLee, CRC Press, ISBN 0-415-27211-4, 147-169.
18. Buckley J, Clapham P. *The Invasion of Helminth Eggs by Chytridiacean Fungi*. J Helminthol. 1929; 7 (1):1-14.
19. Sobenina GG. (1978). *Study of the effect of some fungi on the embryogensis and survival of Ascaris ova*. In: Helminthology Abstract Series A, 47 (10): 440.
20. Lysek H, Bacovsky J. *Penetration of ovicidal fungi into altered eggs of Ascaris lumbricoides*. Folia Parasitol (PRAHA). 1979; 26:139-142.
21. Asitinskaya SF (1979) *The role of freshwater gastropods in removing Ascaris eggs from water*. Abstr. Helminthology. Abstr. Ser. A 48, 89.
22. USEPA-US Environmental Protection Agency. 2003. Control of Pathogens andVector Attraction in Sewage Sludge. EPA 815-R-06-002.
23. WHO (World Health Organization), (2006) Guidelines for the Safe Use of WastewaterExcreta and Greywater, vol. 4. World Health Organization, Geneva.
24. Maya C, Torner-Morales FJ, Lucario ES, Hernández E, Jiménez B. *Viability of six species of larval and non-larval helminth eggs for differentconditions of temperature, pH and dryness*. Water Res. 2012; 46:4770-4782.
25. Katakam KK, Mejer H, Dalsgaard A, Kyvsgaard NC, Thamsborg SM. *Survival of Ascaris suum and Ascaridia galli eggs in liquid manure at differentammonia concentrations and temperatures*. Vet Parasitol. 2014; 204:249-257.
26. Bowman DD, Little MD, Reimers RS. *Precision and accuracy of an assay for detecting Ascaris eggs in various biosolid matrices.* Water Res. 2003; 37:2063-2072.
27. Yanko WA (1987) *Occurrence of Pathogens in Distribution and MarketingMunicipal Sludges*. EPA-600 / 1-87-014 National Technical Information Service, Springfield, Virginia.
28. Benti G, Gemechu F. *Parasitic contamination on vegetables irrigated with Awash River in selected farms Eastern Showa, Ethiopia*. J Parasitol Vector Biol. 2014; 6:103-109.
29. De Victorica J, Galvan M. *Preliminary testing of a rapid coupled methodology for quantitation / viability determination of helminth eggs in raw and treated wastewater.* Water Res. 2003; 37:1278-1287.
30. Karkashan A, Khallaf B, Morris J, Thurbon N, Rouch D, Smith SR, Deighton M. *Comparison of methodologies for enumerating and detecting the viability of Ascaris eggs in sewage sludge by standard incubation-microscopy, the BacLight Live / Dead viability assay and other vital dyes*. Water Res. 2015; 68:533-544.
31. Oksanen A, Eriksen L, Roepstorff A, Ilsoe B, Nansen P, Lind P. *Embryonation and infectivity of Ascaris-suum eggs ea cmparison of eggscollected from worm uteri with eggs isolated from pig feces.* Acta Vet Scand. 1990; 31:393-398.
32. Ayres RM, Mara DD. (1996) *Analysis of wastewater for use in agriculture. A laboratory manual of parasitological and bacteriological techniques*. World Health Organization, Geneva, Switzerland.
33. Amoah ID, Singh G, Stenström TA, Reddy P. *Detection and quantification of soil-transmitted helminths in environmental samples: A review of current state-of-the-art and future perspectives*. Acta Trop. 2017; 169:187-201.
34. Gomez-Couso H, Freire-Santos F, Amar CFL, Grant KA, Williamson K, Ares-Mazas ME, McLauchlin J. *Detection of Cryptosporidium and Giardia in molluscan shellfish by multiplexed nested-PCR.* Int J Food Microbiol. 2004; 91:279-288.
35. Ishiwata K, Shinohara A, Yagi K, Horii Y, Tsuchiya K, Nawa Y. *Identification of tissue-embedded ascarid larvae by ribosomal DNA sequencing.* Parasitol Res. 2004; 92:50-52.
36. Van Frankenhuyzen JK, Trevors JT, Lee H, Flemming CA, Habash MB. *Molecular pathogen detection in biosolids with a focus on quantitative PCR using propidium monoazide for viable cell enumeration*. J Microbiol Methods. 2011; 87:263-272.
37. Reimers RS, Little MD, Englander AJ, Leftwich DB, Bowman DD, Wilkinson RF (1981). *Parasites in southern sludges and disinfection by standard sludge treatment*. EPA 600 / 2-81-160.
38. Bass D, Stentiford GD, Littlewood DTJ, Hartikainen H. *Diverse applications of environmental DNA methods in parasitology.* Trends Parasitol. 2015; 31:499-513.
39. Valero MA, Perez-Crespo I, Periago MV, Khoubbane M, Mas-Coma S. *Fluke egg characteristics for the diagnosis of human and animal fascioliasis by Fasciola hepatica and F. gigantica.* Acta Trop. 2009; 111:150-159.
40. Sultana Y, Jeoffreys N, Watts MR, Gilbert GL, Lee R. *Real-time polymerase chain reaction for detection of Strongyloides stercoralis in stool*. Am J Trop Med Hyg. 2013; 88:1048-1051.
41. Jordanova D, Harizanov R. (2012) *Study of the prevalence of parasitic infections among the population of Bulgaria transmitted through contaminated food and water for the period 1995-2009*. Vth National Scientific Conference of the Bulgarian Focal Point of EFSA, Abstract book: pp. 13-14.
42. Georgieva DA, Prelesov P, Koynarski V. *Parasitological study of soil and sand samples from different regions of Stara Zagora.* Bulg J Vet Med. 1999; 2(2-3):125-130.
43. Georgieva D, Kirkova H, Ivanov A, Chakurova B, Chakurova P, Freedman P, Petkov D. *Induced morbidity in children. Parasitological study of playgrounds in the city Stara Zagora.* Pediatrics. 2005; 35:23-26.
44. Muhtarov M, Rainova I, Tamarozzi F. *Treatment of Hepatic Cystic Echinococcosis in Patients from the Southeastern Rhodope Region of Bulgaria in 2004–2013: Comparison of Current Practices with Expert Recommendations*. Am J Trop Med Hyg. 2016; 94(4):900-905.
45. Popova-Daskalova G. (2018). "*Clinical, Diagnostic and Therapeutic Studies of Cystic Echinococcosis*" PhD Thesis. Medical University Plovdiv.
46. Videnova M, Tsvetkova N, Borisova R, Raynova I, Harizanov R, Ivanova A. (2018) *Sanitary-parasitological studies for the presence of parasitic protozoans and helminths in samples from Sofia and other regions of Bulgaria*. 16th National Congress of Clinical Microbiology and Infections of the Bulgarian Association of Microbiologists 16-18 April 2018, Sofia. Abstract book, p. 65.

STUDY OF THE DISTRIBUTION OF PNEUMOCYSTOSIS IN BULGARIA BETWEEN SEPTEMBER 2017 AND APRIL 2019 BY USING REAL-TIME PCR

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ABSTRACT

Pneumocystis jirovecii is an opportunistic organism that inhabits predominantly the human pulmonary alveoli. The fully sequenced genome of *P. jirovecii* was first reported in 2012. According to some authors, *P. jirovecii* is an obligate pathogen because genes encoding virulence factors and most enzymes for amino acid biosynthesis were not found in the genome. This suggests that the microorganism normally colonises the human lungs but causes disease only in immunocompromised persons. Pneumocystis pneumonia (PCP) is one of the most common opportunistic infections in patients developing acquired immune deficiency syndrome (AIDS). The use of conventional microscopic methods in diagnosis is associated with considerable limitations. Therefore, detection of *Pneumocystis* DNA in clinical samples by PCR techniques leads to significant advances in the diagnosis of PCP.

The aim of this study is to determine the importance of PCR-based methods in the diagnosis of human pneumocystosis and to evaluate their diagnostic value in comparison with conventional microscopy methods. For a period of 20 months in the National Reference Laboratory "Diagnosis of Parasitic Diseases" at the National Centre of Infectious and Parasitic Diseases 33 patients were tested by real-time PCR and 11 of them were found positive for the presence of *P. jirovecii* DNA. Eight of the patients (72.7%) were HIV-infected. Although limited in extent, this is the first real-time PCR study on the distribution of human pneumocystosis in Bulgaria. Our data shows that PCR techniques have higher sensitivity and specificity than microscopic methods and provide new opportunities for the diagnosis of *Pneumocystis* pneumonia.

KEYWORDS:

Pneumocystis jirovecii, real-time PCR, compromised immunity

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INTRODUCTION

In the 1940s of the 20th century, *Pneumocystis* was first recognised as a pathogen causing pneumonia in malnourished or prematurely born children. Before the 1980s, *Pneumocystis jirovecii* pneumonia (PCP) was diagnosed mainly in persons with malignant haematological diseases (1). Following the outbreak of the global human immunodeficiency virus (HIV) epidemic, the incidence of PCP significantly increased. The introduction of antiretroviral therapy and prophylaxis led to a decrease in PCP morbidity in HIV-positive individuals, but still pneumocystis pneumonia is one of the most common opportunistic infections in patients developing acquired immune deficiency syndrome (AIDS) (2, 3).

Pneumocystis spp. are single-celled organisms that can complete their life cycle in the lungs of many mammals (4). *Pneumocystis jirovecii* lives predominantly in the human pulmonary alveoli. Morphological studies have revealed three distinct forms: trophozoite (trophic form), often forming clusters; sporozoite (precystic form) and the cyst which contains several intracystic bodies (spores) (5). The trophic form, adhering tightly to the alveolar epithelial cells type I, is with diameter of 1-4 μm, and the mature cyst is 8-10 μm in diameter. During lung infection in humans, the ratio between trophic and cystic forms is approximately 10:1 (6, 7).

Initially *Pneumocystis* was classified as a protozoan organism because of the morphological characteristics of the two identified forms in the life cycle – the small trophozoite and a larger cyst form, and also because of the response of *Pneumocystis jirovecii* infection to treatment with antiprotozoal drugs (5). Based on DNA sequence analysis, *Pneumocystis* is currently classified as a fungus, although it is difficult to cultivate in a standardised system. The fully sequenced genome of *P. jirovecii* was first reported in 2012 (8). Genes encoding virulence factors and most enzymes for the biosynthesis of amino acids were not found in the genome. This suggests that the microorganism normally colonises the human lungs but causes disease only in immunocompromised persons. Although the whole genome analysis is completed, explanation of the life cycle and sensitivity to drugs is hampered by the difficulty in isolating *Pneumocystis* in pure culture (3, 5). A major problem in the evaluation of diagnostic tests for PCP is the lack of a golden standard, mainly because there is no system for culturing *P. jirovecii* (9). There are serious limitations in the sensitivity of microscopy diagnosis, the low-level invasion particularly in non-HIV-infected patients, and also in using non-invasive methods for collection of pulmonary specimens (10). Stained smears for microscopy diagnosis are most often prepared from Gomori's methenamine silver (GMS), Calcofluor white, Giemsa, toluidine blue and commercially available specific *Pneumocystis* immunofluorescent diagnostic kits (11, 12).

Detection of *Pneumocystis* DNA in clinical specimens by using polymerase chain reaction (PCR) analysis led to a significant advance in the diagnosis of PCP (9). This is the most sensitive method for detection of *Pneumocystis* and should be considered as the first choice among diagnostic tests. In fact, real-time PCR is currently regarded as the main tool for diagnosing PCP (10, 11).

Bronchoalveolar lavage fluid is the optimal clinical specimen type for PCR analysis but induced sputum is also acceptable, especially in HIV-infected patients. PCR assays have showed that *Pneumocystis* DNA can be detected in oropharyngeal washes and nasopharyngeal aspirates (3, 6).

The aim of this research is to determine the importance of PCR-based methods in the diagnosis of human pneumocystosis and to evaluate their diagnostic value in comparison with conventional microscopy methods.

MATERIAL AND METHODS

Patients

For a period of 20 months, in the National Reference Laboratory "Diagnosis of Parasitic Diseases" at the National Centre of Infectious and Parasitic Diseases, were tested 33 patients suspected for *Pneumocystis* pneumonia. Eleven were with HIV infection. From all 33 tested patients, 14 were children and adolescents between 0-19 years and 19 were adults. Gender distribution showed that 8 of the patients (24.2%) were female and 25 (75.8%) – male.

Clinical samples

During the study period the following samples were tested: bronchoalveolar lavage fluid (n = 2), tracheal aspirate (n = 2), induced sputum (n = 28), post-mortem material from lungs (n = 1).

Microscopic diagnosis

From 15 of the obtained samples were prepared smears stained by the Romanovsky-Giemsa method and toluidine blue. Microscopy was performed with magnification of 10x100.

DNA isolation and real-time PCR

All 33 samples were subjected to DNA analysis. DNA was extracted according to the manufacturer's protocol using the commercial kit PureLink® (Genomic DNA Kits, Invitrogen, Life technologies) based on the selective binding to silica membranes in the presence of chaotropic salts. Real-time PCR analysis was performed by using the commercial kit RIDA®GENE *Pneumocystis jirovecii* (R-Biofarm AG) with amplification of a DNA fragment of the mitochondrial large subunit gene, specific for *P. jirovecii*. The kit contains internal control that monitors PCR inhibition and confirms whether nucleic acid extraction is successful. The reaction was carried out in LightCycler®480 II, Roche.

RESULTS

For a period of 20 months a total of 33 samples were tested by real-time PCR for the presence of

P. jirovecii DNA. Twenty-two samples were from patients without established HIV infection or AIDS, from immunocompetent or patients with other form of immunosuppression, and 11 were from HIV-infected persons (Table 1).

In the group of HIV-positive patients we found 8 positive samples (72.7%). Among HIV-negative patients there was a significantly fewer number of positive results – 3 (13.6%). *P. jirovecii* DNA was found in clinical materials from induced sputum (n = 10) and tracheal aspirate (n = 1).We prepared stained smears from 15 samples for light microscopy diagnosis and results were negative. The same 15 samples were tested by real-time PCR and 12 of them were negative, but in 3 there was amplification of the target sequence.

The affected persons were aged from 0 to 49 years. The largest number of cases was in the age group 30-34 years, followed by 0-4 years and 45-49 years (Table 2).

Table 1. Distribution of pneumocystosis cases by primary diagnosis.

Primary diagnosis	Number of cases	Positive for *P. jirovecii* DNA	Positive cases in %
HIV	11	8	72.7
Following liver transplantation	2	0	0
Patients with severe pneumonia	17	3	17.6
Other (respiratory distress syndrome, death of a newborn, nephrotic syndrome with acute respiratory failure)	3	0	0

Table 2. Distribution of cases by age group.

Age group	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	75-79
Number of tested persons	8	0	0	6	1	3	4	4	1	3	1	0	1	1
***P. jirovecii* DNA-positive persons**	2	0	0	1	0	1	3	1	1	2	0	0	0	0

DISCUSSION

Transmission of *P. jirovecii* in the human population is airborne. There is an assumption that about 95% of people are infected during childhood but healthy adults are asymptomatic carriers. The disease usually develops in cases of compromised immunity. The prevalence of HIV-associated *P. jirovecii* pneumonia varies worldwide: in Europe it is about 16%, in Africa reaches 39% and in Southeast Asia (Malaysia) – up to 63% (2, 13). Data on the prevalence of *P. jirovecii* in Bulgaria are limited. Kurdova et al. (2004) reported results of an 11-year study (1993-2003) on opportunistic parasites causing morbidity in HIV-infected persons. Among the 165 patients examined with microscopy 10 (6.06%) were with positive results for pneumocystosis (14). Our study showed significantly larger percentage of pneumocystosis cases among HIV-infected people (72.7%). We believe that this is due to the higher sensitivity of PCR techniques in general, as well as the small number of examined patients and the shorter study period. Our data suggest that besides in HIV-infected, the disease is registered also in other cases of compromised immunity: 4- and 6-months-old infants (n = 2) and a boy at the age of 15 years who developed PCP in association with congenital

agranulocytosis and massive immunosuppressive therapy. In this respect, our data correlate with data in the literature. Ten of the patients with *P. jirovecii* infection were male (90.9%) and only 1 person was an HIV-positive female.

According to the Centres for Disease Control and Prevention (CDC, USA), *P. jirovecii* infection mortality in untreated immunocompromised patients is 100% and is reduced to 5-40% with aetiological treatment (15). Therefore, it is essential to determine the aetiological diagnosis promptly and initiate treatment. According to literature data, the type of clinical specimen and choice of diagnostic method are important factors for a better diagnosis.

Laboratory diagnosis of PCP using bronchoalveolar lavage fluid exhibits sensitivity of 98% or higher and this is the preferred material, although sensitivity with induced sputum (50-90%) is also acceptable (3, 16). According to some authors, PCR methods have the highest detection limits, whereas for microscopic methods they are significantly lower. According to Doyle et al. (2017), sensitivity of toluidine blue staining is between 71.4% and 85.7% depending on the type of clinical specimen, for histology – 71.4%-75% and surprisingly low for cytology – 43%. As for PCR, data shows 100% sensitivity. The same team established that PCR-based methods have negative predictive value of 100% and positive predictive value of 93.1% (11). Therefore, it is not uncommon to detect the presence of *P. jirovecii* DNA with real-time PCR, even though microscopic examination shows negative result (16). Data obtained from our survey, despite being limited, confirms this fact.

CONCLUSIONS

Although limited in extent, this is the first real-time PCR study on the distribution of human pneumocystosis in our country. PCR methods provide new opportunities for the diagnosis of *Pneumocystis* pneumonia with higher sensitivity and specificity than microscopic methods. PCR techniques also enable detection of *P. jirovecii* even at low pathogenic load compared to conventional microscopic methods. The small amount of pathogens in the clinical material may be due both to the level of immunosuppression and the initial stage of disease. Early diagnosis and initiation of treatment improves the chances of a favourable patient outcome.

REFERENCES

1. Kovacs JA, Masur H. *Evolving health effects of Pneumocystis: one hundred years of progress in diagnosis and treatment*. JAMA. 2009; 301:2578-2585.
2. Huang L, Cattamanchi A, Davis JL, Boon S, Kovacs J, Meshnick S, Miller RF, Walzer PD, Worodria W, Masur H. *HIV associated Pneumocystis pneumonia*. Proc Am Thorac Soc. 2011; 8(3):294-300.
3. Tasaka S. *Pneumocystis pneumonia in Human Immunodeficiency Virus-infected adults and adolescents: Current concepts and future directions*. Clin Med Insights Circ Respir Pulm Med. 2015; 9(Suppl 1):19-28.
4. Song Y, Ren Y, Wang X, Li R. *Recent Advances in the Diagnosis of Pneumocystis Pneumonia*. Med Mycol J. 2016; 57(4):111-116.
5. Gigliotti F, Limper AH, Wright T. *Pneumocystis*. Cold Spring Harb Perspect Med. 2014; 4:a019828.
6. Carmona EM, Limper AH. *Update on the diagnosis and treatment of Pneumocystis pneumonia*. Ther Adv Respir Dis. 2011; 5:41-59.
7. Calderón EJ, Gutiérrez-Rivero S, Durand-Joly I, Dei-Cas E. *Pneumocystis infection in humans: diagnosis and treatment*. Expert Rev Anti Infect Ther. 2010; 8:683–701.
8. Cissé OH, Pagni M, Hauser PM. *De novo assembly of the Pneumocystis jirovecii genome from a single bronchoalveolar lavage fluid specimen from a patient*. MBio. 2012; 4:e428-12.
9. Moodley B, Tempia S, Frean JA. *Comparison of quantitative real-time PCR and direct immunofluorescence for the detection of Pneumocystis jirovecii*. PLoS ONE. 2017; 12(7):e0180589.
10. Sasso M, Chastang-Dumas E, Bastide S, Alonso S, Lechiche C, Bourgeois N, Lachaud L. *Performances of four real-time PCR assays for diagnosis of Pneumocystis jirovecii pneumonia*. J Clin Microbiol. 2015; 54:625-630.
11. Doyle L, Vogel S, Procop GW. *Pneumocystis PCR: It Is Time to Make PCR the Test of Choice*. Open Forum Infect Dis. 2017; 4(4):ofx193.
12. Kurdova R. (ed.) *Laboratory diagnosis of parasitic infections in humans*. Sofia, "Arso", 2009, 254 p. (in Bulgarian)
13. Ding CH, Yusoff H, Muttaqillah NAS, Tang YL, Tan TL, Periyasamy P, Ban AYL. *The crucial role of molecular testing to facilitate the diagnosis of pneumocystis pneumonia during pregnancy*. Malaysian J Pathol. 2018; 40(1):69-72.
14. Kurdova R, Marinova T, Jordanova D, Ivanova M, Tsvetkova N, Rainova I. *Opportunistic Parasitic Diseases Associated with HIV Infection in Bulgaria*. EMOP IX, 2004.
15. Centers for Disease Control and Prevention. Pneumocystis pneumonia Statistics 2012.
16. Fillaux J, Malvy S, Alvarez M, Fabre R, Cassaing S, Marchou B, Linas MD, Berry A. *Accuracy of a routine real-time PCR assay for the diagnosis of Pneumocystis jirovecii pneumonia*. J Microbiol Methods. 2008; 75(2):258-261.

SURVEILLANCE DATA ON BACTERIAL ENTEROCOLITIS IN BULGARIA FOR 2014-2018

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ABSTRACT

Worldwide, 30% of cases of infectious diarrhoea are caused by bacterial pathogens. As in other countries of the European Union, in Bulgaria the most common etiological agents are *Escherichia coli* (STEC), *Salmonella* spp.*, Shigella* spp.*, Campylobacter* spp. *and Yersinia enterocolitica*. Acute gastroenteritis and enterocolitis infections are most common in young children. According to our survey, the leading pathogens for 2014-2018 are *Salmonella* spp.*, E. coli* (EPEC, ETEC) and *Campylobacter* spp. The rate of infections caused by *Shigella* spp. is relatively high compared to other European countries.

The number of enterocolitis cases of undefined aetiology continues to increase because of the neglect towards diarrhoeal syndrome by patients who rarely visit a doctor or do not seek medical attention at all.

KEYWORDS:

enterocolitis, *Salmonella* spp.*, E. coli, Campylobacter* spp.*, Shigella* spp.

INTRODUCTION

Acute infectious diarrhoea has a major role in infectious pathology, with the most affected groups being young children and immunosuppressed patients. Worldwide, there are around 1.5 billion cases of acute enterocolitis every year. The etiological structure of acute infectious diarrhoea varies among different age groups and geographic regions. Bacterial pathogens account for 30% of cases of infectious diarrhoea and the most common etiological agents are *E. coli* (STEC), *Salmonella* spp.*, Shigella* spp.*, Campylobacter* spp. and *Yersinia enterocolitica* (1-3, 6).

This report describes surveillance data on laboratory-diagnosed infections caused by seven foodborne or waterborne enteric bacterial pathogens for the last five years in Bulgaria.

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MATERIAL AND METHODS

Infection cases, incidence and trends

In Bulgaria over the last five years intestinal infections account for 42.27% of registered cases of acute infectious diseases. Acute enterocolitis and gastroenteritis comprise 81.38% of all intestinal diseases and are most common in young children (4, 6). The etiological structure of acute infectious diarrhoea caused by bacterial pathogens is presented in Fig. 1. Bacteriological confirmation of the disease relies on isolation of the organism from stool samples. The distribution of bacterial isolates in cases of acute infectious diarrhoea in Bulgaria is presented in Fig. 2-7.

Salmonella species are the leading bacterial agents of enterocolitis in Bulgaria. A total of 1780 strains of *Salmonella enterica* were confirmed at the National Reference Laboratory (NRL) of Enteric Pathogens. For the last five years the most commonly found serotypes were: *S.* Enteritidis – 52.25%, *S.* Typhimurium – 12.36%, *Salmonella* 1,4,[5],12:i:- – 11.24%, *S.* Derby – 7.30%, *S.* Infantis – 5.9%, *S.* Schleissheim – 2.95%, other serotypes – 8%. These results coincide with other data on the most frequently isolated *Salmonella* spp. in Bulgaria (Enteritidis and Typhimurium) (5). Serotype distribution is presented in Fig. 8.

In the last five years have been reported several foodborne outbreaks involving *S.* Enteritidis. There were three outbreaks in the cities of Ruse and Varna in 2015, also Pleven and Veliko Tarnovo – in 2016, Stara Zagora – in 2018 and every year in the city of Sofia. In 2018 was reported an outbreak caused by the previously not described in Bulgaria *S. enterica* subsp. *enterica* serovar London, affecting only medical staff in two healthcare establishments. Furthermore, since 2016 culture isolation of single-phase *Salmonella* Typhimurium (1,4,[5],12:i:-) has been significantly increasing.

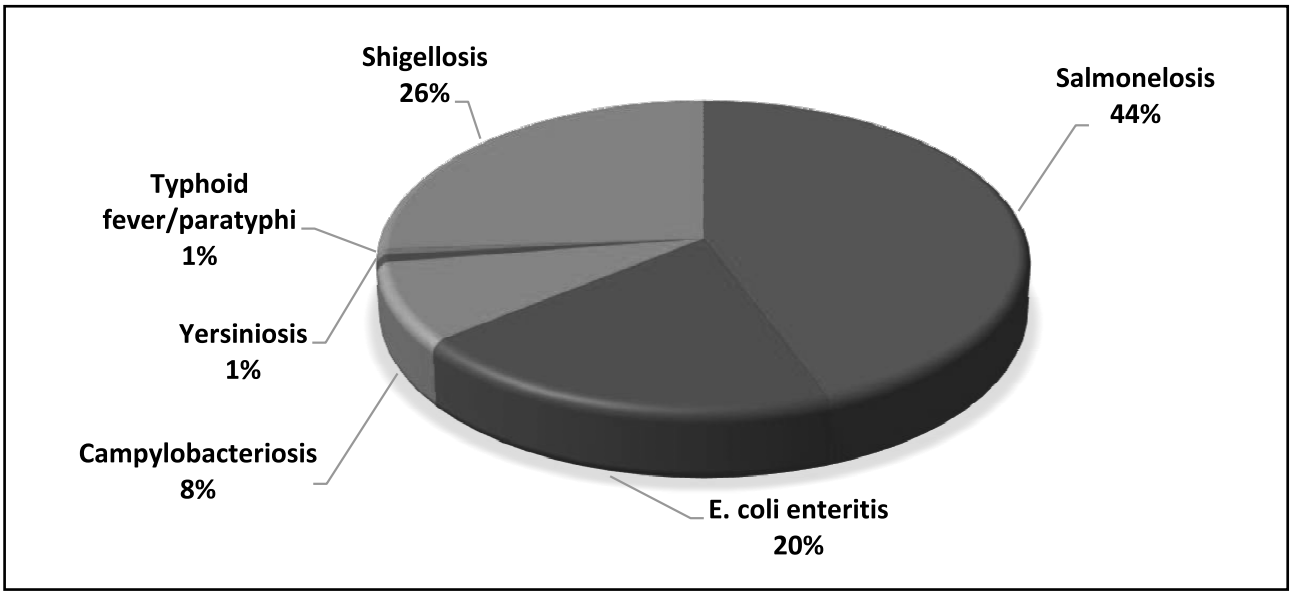

Figure 1. Etiological structure of acute infectious diarrhoea caused by bacterial pathogens over the last five years 2014 - 2018.

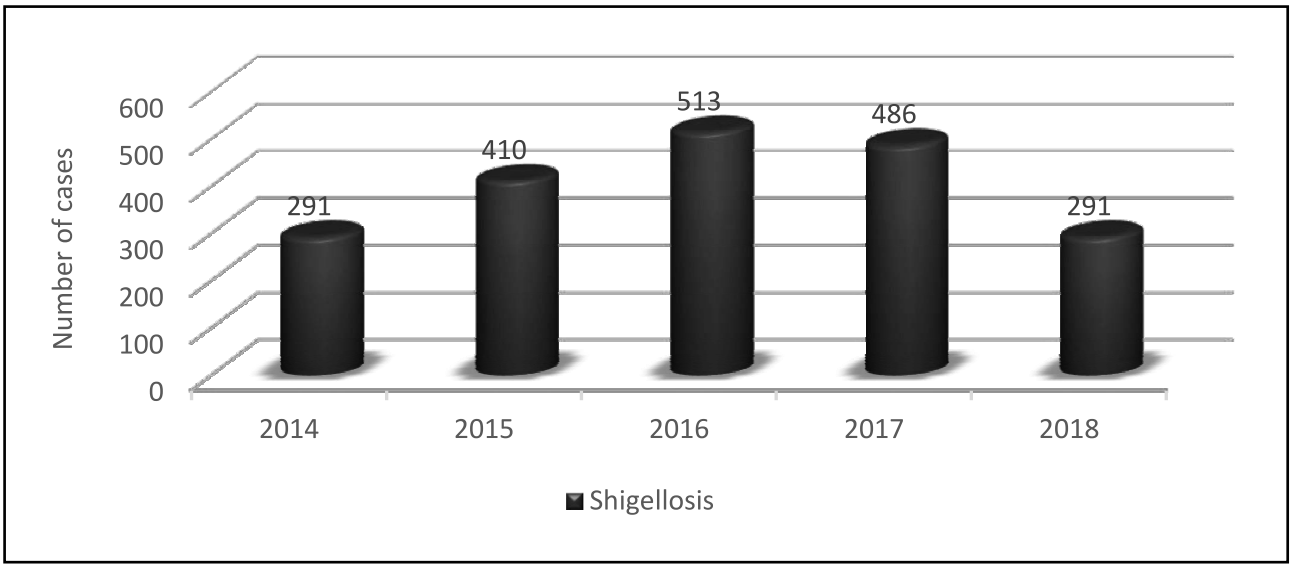

Figure 2. Distribution of shigellosis 2014-2018.

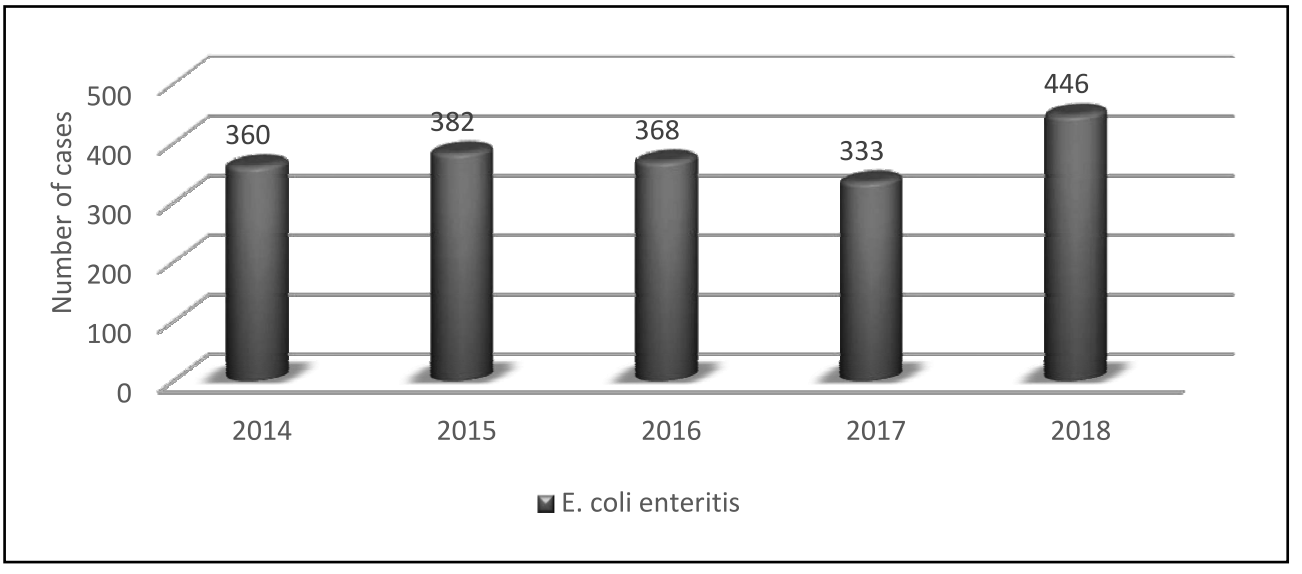

Figure 3. Distribution of *E. coli* enteritis 2014-2018.

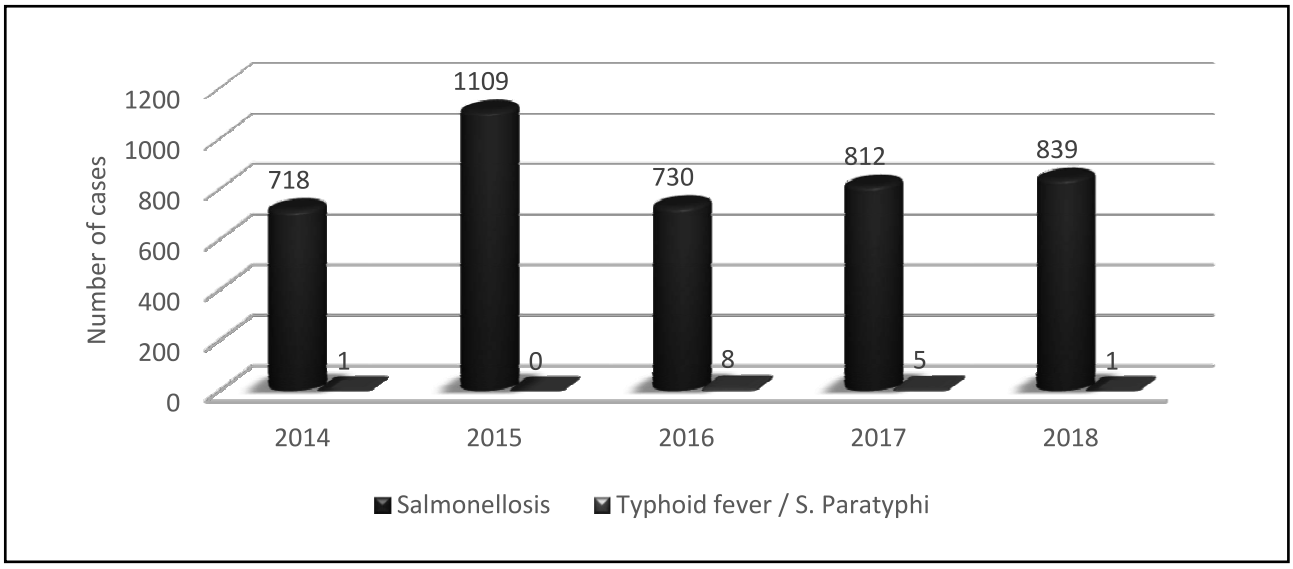

Figure 4. Distribution of salmonellosis and typhoid fever 2014-2018.

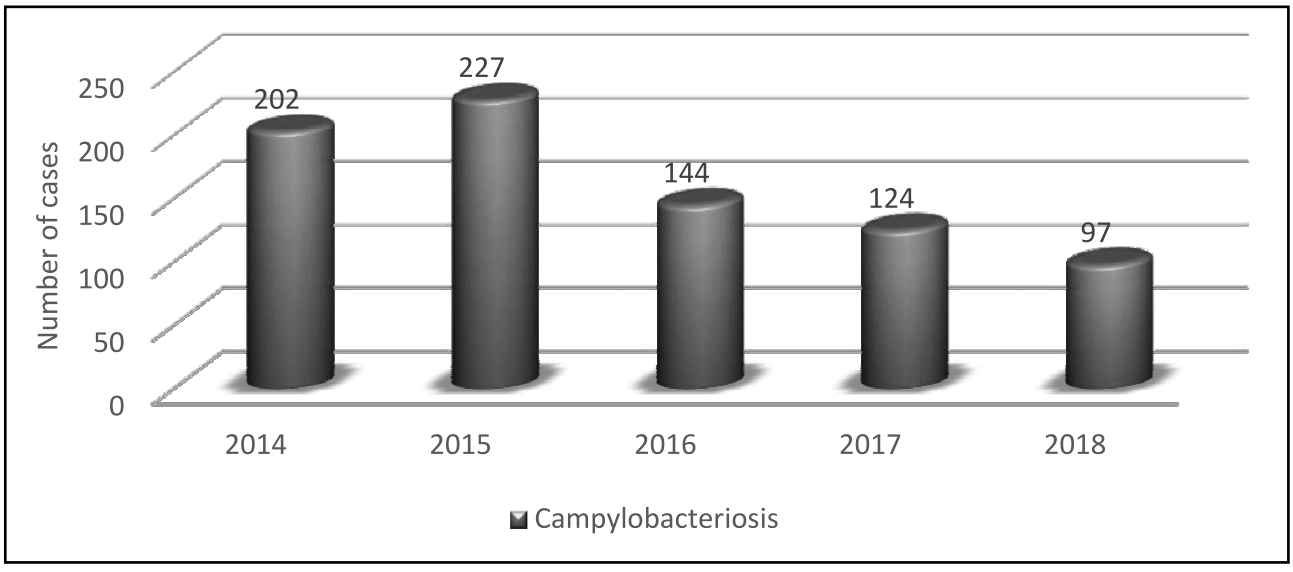

Figure 5. Distribution of campylobacteriosis 2014-2018.

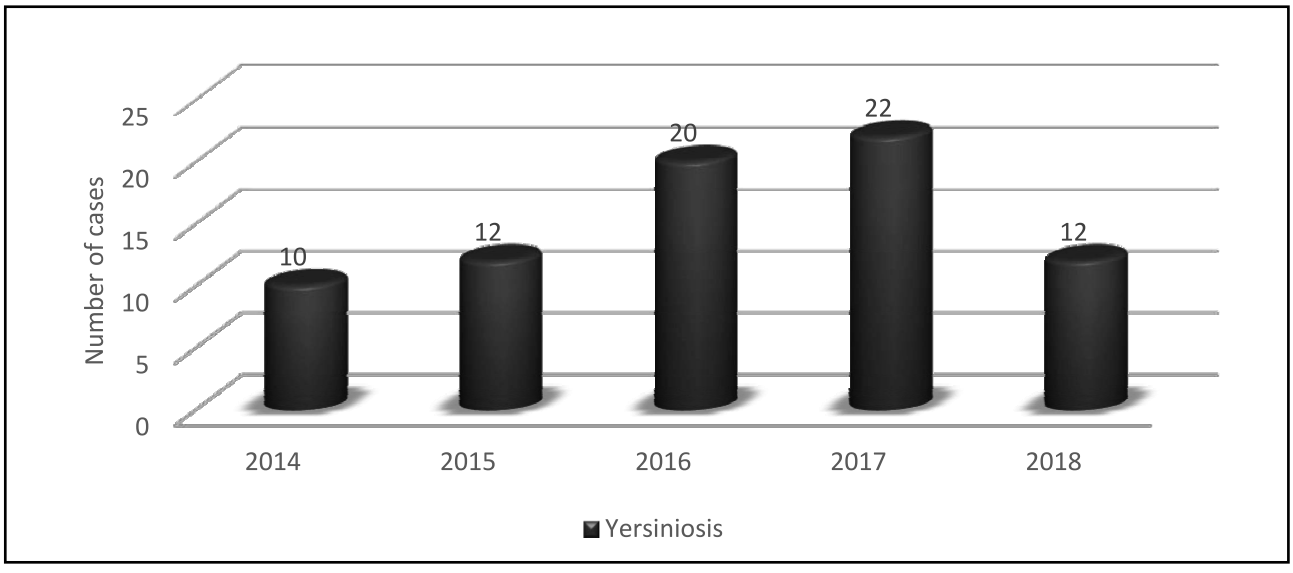

Figure 6. Distribution of yersiniosis 2014-2018.

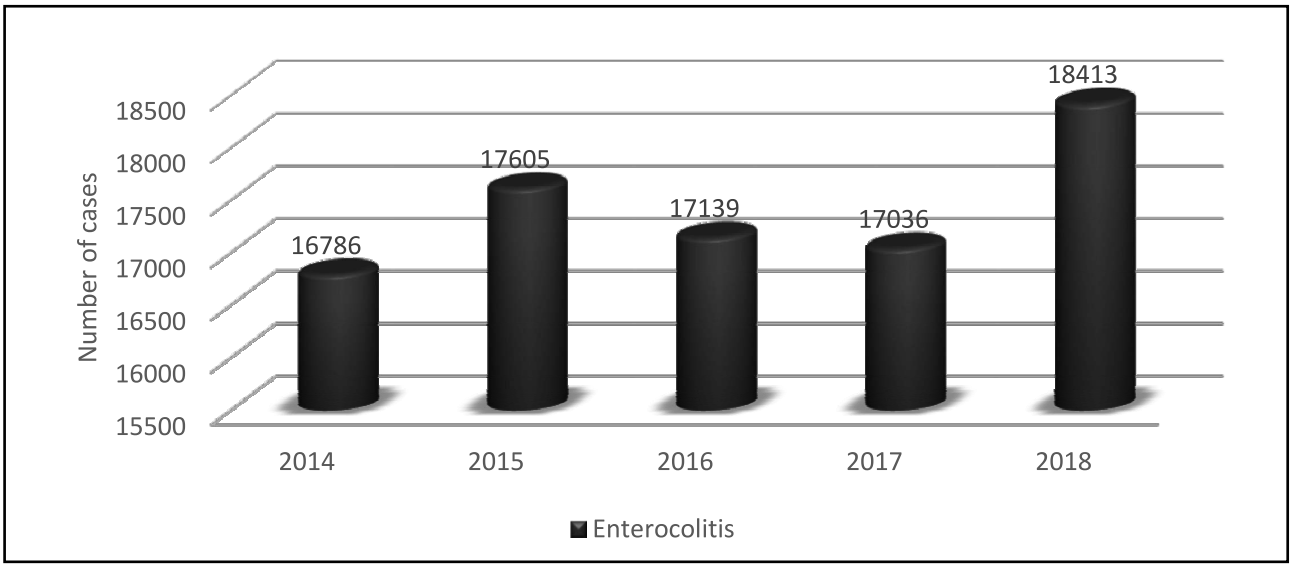

Figure 7. Distribution of enterocolitis 2014-2018.

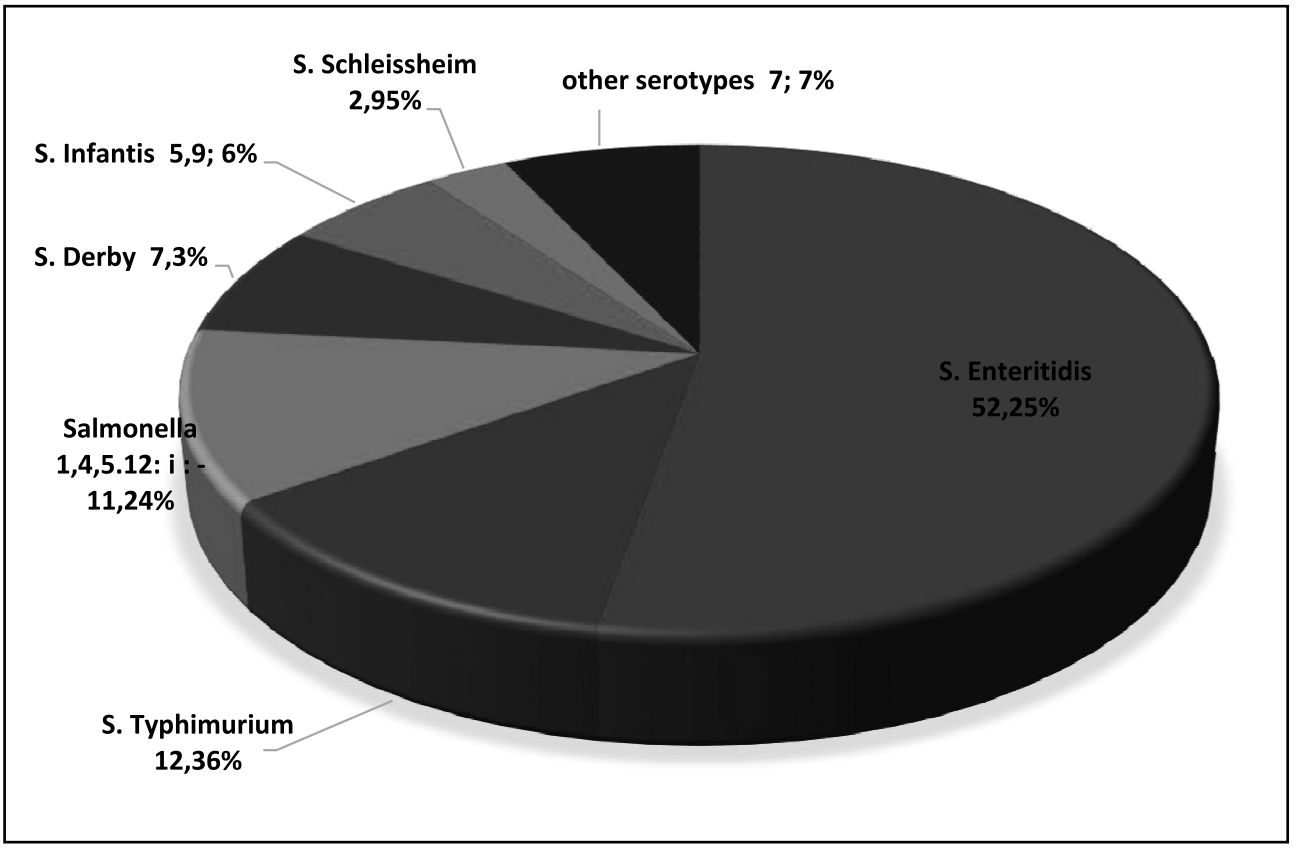

Figure 8. Distribution of *Salmonella* serotypes in Bulgaria 2014-2018.

Typhoid fever is an infectious disease occurring primarily in developing countries, while in developed countries it is still confined to returning travellers or contacts of patients (8, 9). There have been a few cases of *Salmonella* Typhi over the last five years – sporadic cases in patients arrived from abroad (India, Pakistan, Thailand, etc.) and an epidemic outbreak affecting six patients in a hospice in 2014.

The recently increasing number of human isolates of *S. enterica* subsp. *enterica* serovar Paratyphi B biovar Java is often associated with illnesses manifesting as prolonged fever with or without other systemic symptoms. All Paratyphi isolates were confirmed as biovar Java by a PCR-based method at the NRL of Enteric Pathogens, NCIPD.

Although *Salmonella* is the most commonly reported causative agent of bacterial enterocolitis, the number of enterocolitis infections of undefined aetiology continues to increase.

In our country, shigellosis morbidity is higher compared to other European countries. The leading etiological agents are *Shigella flexneri* – 80%, followed by *Shigella sonnei* – 16% and *Shigella boydii* – 2%. Most cases of shigellosis affect minority populations. The underlying sanitary and hygienic conditions are indicated as the main factor for the spread of infection.

E. coli enteritis is most common in infants and young children. According to the database collected at the NRL of Enteric Pathogens, the leading etiological agents are EPEC O6 in 55% of the cases, followed by EPEC O127 and O44 (Fig. 9). To date, there is no registered case of infection caused by Shiga/Vero toxin-producing *E. coli* in Bulgaria.

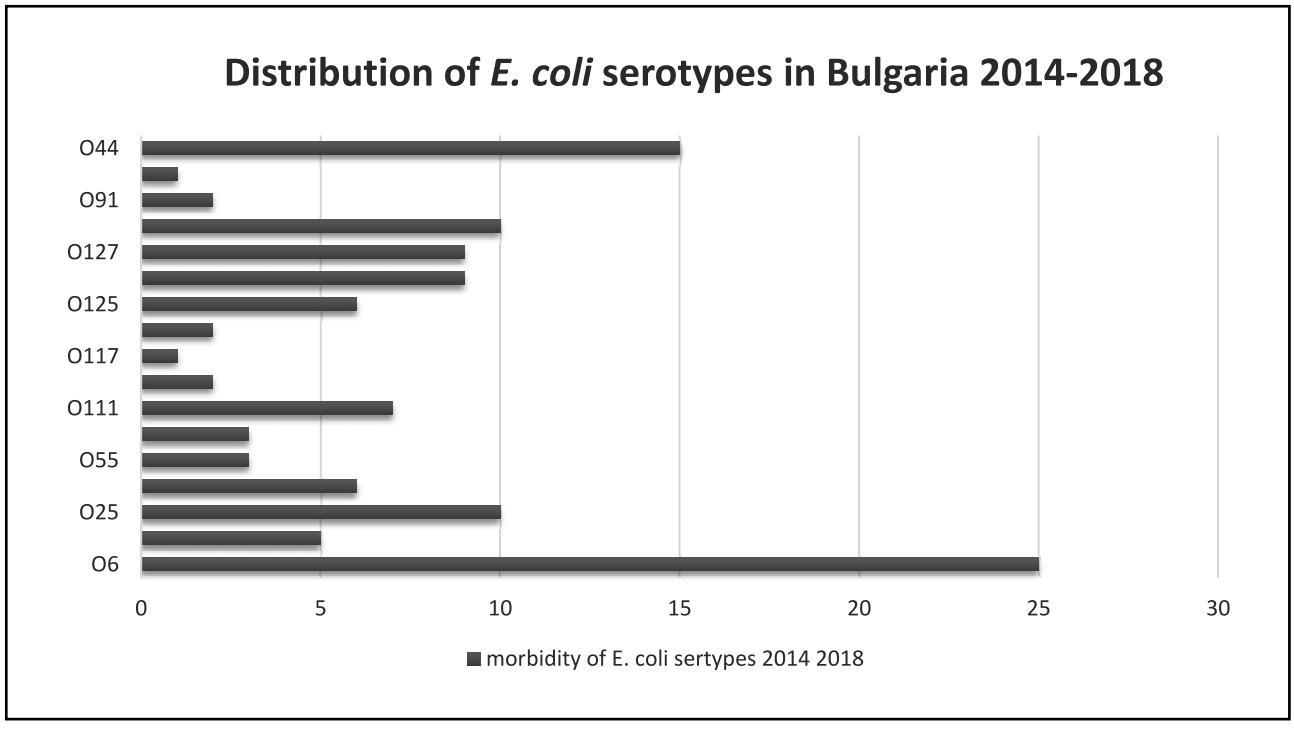

Figure 9. Distribution of E. coli serotypes in Bulgaria 2014-2018.

All registered cases of yersiniosis were laboratory-confirmed with the isolation of *Y. enterocolitica*. There is a steady trend in the reporting of sporadic cases mostly in immunosuppressed patients or those with concomitant illnesses.

The incidence of *Campylobacter* infections, taking into account only cases with positive culture results, was significantly lower in Bulgaria compared to other EU countries (7). A confirmed bacterial infection is defined as isolation of the bacterium from a clinical specimen by culture. However, pathogen detection could be affected by laboratory testing practices. The unwillingness to perform laboratory diagnosis impedes the determination of the actual morbidity due to campylobacteriosis which is a leading disease among foodborne infections in the EU countries. Pathogen detection could be enhanced if clinical laboratories adopt DNA-based tests (automated systems) as they are quicker and easier to perform than traditional culture methods. Also laboratories could examine pathogens that are not often included in the routine stool culture. Nevertheless, it should be noted that each year there is an increase in the number of *Campylobacter* clinical isolates sent for microbiological confirmation to the NCIPD.

RESULTS

Routine stool cultures performed in clinical laboratories typically include methods that identify only *Salmonella*, *Campylobacter*, *Shigella*, *E. coli* (EPEC, ETEC, EIEC) and one of the Shiga toxin–producing types of *E. coli* O157 for some laboratories.

In the last five years, intestinal infections comprise 31.02% of the recorded acute cases of infectious diseases in the country. Acute gastroenteritis and enterocolitis remain

the leading infectious intestinal diseases – 81.38%. There is an increased incidence of bacterial enterocolitis during the summer months and the most affected group is young children. Salmonellosis remains the leading bacterial intestinal infection in Bulgaria followed by *E. coli* (EPEC, ETEC).

CONCLUSION

The number of enterocolitis cases of undefined aetiology continues to increase because of the neglect towards diarrhoeal syndrome by patients who rarely visit a doctor or do not seek medical attention at all.

DATA SOURCES:

- National Centre of Public Health and Analyses (NCPHA) – Sofia, Bulgaria;
- Regional Health Inspections (RHI) - annual analyses of current diseases;
- National Reference Laboratory for Pathogenic Diseases at the National Centre of Infectious and Parasitic Diseases, Sofia;

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REFERENCES

1. *Preliminary FoodNet data on the incidence of foodborne illnesses-selected sites, US, 2001*. MMWR Morb Mortal Wkly Rep. 2002; 51(15):325-329.
2. EFSA-ECDC annual report on animal infections transmissible to humans. 2011. http://www.efsa.europa.eu/
3. Allen SJ, Martines EG, Gregorio GV, Dans LF. *Probiotics for treating acute infectious diarrhoea*. Cochrane Database Syst Rev. 2010; (11):CD003048.
4. Registered diseases at national level, 2017 http://ncphp.government.bg/index.php?lang=bg
5. Parmakova K, Aseva G, Ivanova K, Petrov P. *Surveillance of Bacterial Intestinal Infections in Bulgaria in 2007-2011*. Medinfo. 2012; 11.
6. Stoicheva M, Velcheva R, Murdjeva M, Petrov M. *Intestinal infections with diarrhea syndrome in children up to 5 years of age*. Medicart. 2010; 3.
7. Campylobacteriosis - Annual Epidemiological Reports. https://ecdc.europa.eu/en/publications-data
8. Joshi S, Wattal C, Sharma A, Prasad KJ. *Mixed Salmonella infection – a case report*. Indian J Med Microbiol. 2002; 20:113-114.
9. Asseva G, Petrov P, Bozova E, et al. *Updates on molecular epidemiology of S. Typhi in Bulgaria with review of outbreak and sporadic cases, 2008-2014*. Probl Infect Parasit Dis. 2015; 43(1):8-12.

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HAPPY ANNIVERSARY!

On October 1st, 2019 Prof. Petar Andonov is celebrating his 100th anniversary. He is a prominent Bulgarian virologist with significant merits for the development of medical virology in this country.

Prof. Peter Andonov, MD was born in 1919 in Kyustendil. He studied medicine in the Medical Faculty of Sofia University. He was the first PhD student from Bulgaria at the Dmitry Ivanovski Institute of Virology in Moscow, Russia. He is the founder of the Virology Department at the National Center for Infectious and Parasitic Diseases in Sofia. The new building of the Department located on 44A Gen. Stoletov Blvd, was erected in 1958 and prof. Andonov was the first head of the department. On his initiative, the Hygienic-Epidemic Stations (HES), now Regional Health Inspectorates (RHI) were established with specialized units for virology research. In 1962 Prof. P. Andonov started for the first time teaching virology at the Faculty of Biology at Sofia University "St. Kliment Ohridski" and it is his contribution the Biological faculty to be a continuous training ground for young virologists. During his professional career, he held a number of responsible positions, including Deputy Minister of Health from 1977 to 1981, Rector of the Higher Medical Institute in Sofia (1981 - 1985) and Editor-in-Chief of the "Health Front" newspaper (1960 – 1966). The first textbook on virology in Bulgarian was also written by Prof. Andonov. He is the author of more than 200 scientific papers and reviews on current problems of virology, as well as more than 15 patents and inventions. Prof. Peter Andonov has established and chaired for fifteen years the Specialized Scientific Council of Virology at the Higher Attestation Commission. He is also one of the co-founders of the First International Committee on Classification and Nomenclature of Viruses in Moscow, Russia. He has also been a longtime expert at the WHO.

Prof. Peter Andonov is among us, enjoying a good health and continuing to be interested in the progress of virology science and the development of the scientific careers of the staff at the Department of Virology at the National Center for Infectious and Parasitic Diseases..

We, the colleagues from the National Center for Infectious and Parasitic Diseases express our congratulations on the occasion of the 100th jubilee birthday of Prof. Peter Andonov, and wish him good health.

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