Cryptosporidium in water and food

Prof. Panagiotis Karanis
Medical School, University of Cologne, Germany
Contents of this presentation

Short self – introduction

*Cryptosporidium* - What are the facts:
- Importance of *Crypto*
- Prevalence & outbreaks
- What is Cryptosporidium?
- Transmission & life cycle
- Crypto in food
- Diagnosis
- Treatment & prevention
- Water treatment & pathogen removal

What are the problems?
- Inter alia tourism in danger

What are the solutions?
- Take home message
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Qinghai University in Xining, China

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XINING

Qinghai

Pics of Qinghai Province
1000 Talent Plan of the Chinese Government

Qinghai University

QINGHAI UNIVERSITY

清海大學
Upgraded to Key State Laboratory for Plateau Ecology and Agriculture
1000 TP of the Chinese Government
Chinese scientist Tu Youyou wins Nobel Prize for breakthrough malaria treatment developed under Chairman Mao

Chinese scientist Tu Youyou won a share of the Nobel Prize in Physiology or Medicine yesterday for her work in developing an effective herbal therapy that fights against malaria, work that first started when studying ancient Chinese texts in a secret project ordered by Chairman Mao Zedong during the Cultural Revolution.

Tu becomes the first Chinese scientist to win a Nobel Prize in the sciences who has spent the majority of his or her career in China. She also becomes China’s first Nobel Prize winner in medicine and just the 12th woman overall to win in that field.

The 85-year-old is famous as the inventor of artemisinin, a highly effective treatment against malaria with unbelievable origins.

Tu’s work on artemisinin dates back to a secret military project known only as “523” directly ordered by Mao during the Cultural Revolution. Its purpose was to develop an efficient anti-malaria drug as the disease had been wiping out North Vietnamese troops fighting in the jungles of southeast Asia. The project cycled through hundreds of scientists and researchers, many of whom were purged after less than a year from political vendettas.

Tu turned out to be the most successful of these researchers and found the answers that the Chairman wanted in ancient Chinese texts. She found mention of sweet wormwood being used to treat malaria. Through further refinement, the team developed a compound that attacked malaria-causing parasites in the blood. Not content with tests done on animals, Tu tested the new drug on herself first.

“The work was the top priority so I was certainly willing to sacrifice my personal life,” the scientist later recalled.

Today, this drug called artemisinin is a key ingredient in frontline treatment for malaria in Africa and Asia. Experts say that it has saved millions of lives. Here’s what the WHO had to say about Tu being honored with the Nobel Prize:

“It is a great tribute to the contribution of the Chinese scientific community in the fight against #malaria... Since 2000, more than 1 billion #artemisinin -based treatment courses have been administered to malaria patients.”

The 85-year-old scientist is still continuing her work in artemisinin at a lab in China Academy of Traditional Chinese Medicine in Beijing. She has been jokingly called a “Three-Without Scientist.” Tu has no doctorate degree, has not studied abroad and hasn’t received membership in the China’s Academy of Sciences. She does have a Nobel Prize though. That’s something.

Contact the author of this article or email tips@shanghailand.com with further questions, comments or tips.
Prof. Dr. Panagiotis Karanis – Short CV

1959: Born in Chrysso / Evritania / Greece
1979: Germany / Studies of Biology & Medicine
1992: PhD in Parasitology / Bonn University / Germany
1999: Habilitation (*Venia legendi*) in Parasitology, Medical School / Bonn University / Germany
2000 - today: Professor at Cologne Medical School / Germany
1999, 2003, 2004: Visiting Professor at the National Research Center for Protozoan Diseases (NRCPD) / Japan
2005 - 2009: Professor, NRCPD / Japan
2014 - 2017: Professor, Center for Biomedicine and Infectious Diseases, Qinghai University / China
What is *Cryptosporidium*?

- Parasite (Protozoon), cause disease called Cryptosporidiosis in humans
- Most common species *Cryptosporidium hominis* & *C. parvum*, other species
- There are 34 different *Cryptosporidium* – species and 62 genotypes

Dormant / transmissive stage: Oocyst

Oocysts with infectious sporozoites:
- Size: 5 μm
- 2 y survival in the environment by 4˚C
THE IMPORTANCE OF CRYPTOSPORIDIUM
Cryptosporidium is responsible for:

- 1 Mio children’s deaths / year
- Human colon cancer
- Transplant rejection
Every year, diarrhea causes the deaths of 800,000 children under five, which is more than that well-recognized child-killer malaria.

Hedstrom L (2015): Trends Parasitology
Occurrence & worldwide outbreaks of Cryptosporidium
Cryptosporidium Prevalence

- Immunosuppressed, HIV-infected and organ transplants.
- Children, in particular 0-2; 2-4 years.
- In industrialized countries: ‘Crypto.’- detection in 2-4% patients with diarrhea. ‘Crypto’.- detection by 1-4% healthy, asymptomatic humans.
- In developing countries the prevalence is higher compared to industrialized countries.
- In Germany: since 2001 registered by RKI: 800 - 1.500 cases with increasing number from July to the end of November.

www.rki.de
Global burden of food-borne / water-borne (diarrheal) diseases currently unknown

- 1.8 million people died from diarrheal diseases (WHO 2005) due to contaminated food and drinking water.

- This is not just a problem of the underdeveloped world.

- About 76 million cases of food-borne diseases, resulting in 325,000 hospitalizations and 5,000 deaths, are estimated to occur each year only in the U.S.A. (Mead et al., 1999).

- Over 200 known microbial, chemical or physical agents can cause illness when ingested.

- Over the last 20 years, at least in the industrialized world, water- and food-borne diseases caused by bacteria, parasites, viruses and prions have significantly moved up the political agenda and generated substantial media attention.
Cryptosporidium is the number 2 cause (after Rota-virus) of diarrhea-associated mortality in children under 5 years of age, worldwide

Kotlof et al. (2013)
From the begin of the last century until 2016, *Cryptosporidium* was responsible for ~60% (524/913) of all reported waterborne outbreaks caused by protozoan parasites.

**Cryptosporidium: outbreaks**

**Largest waterborne Cryptosporidium outbreak:**
- Milwaukee, USA in 1993 (1)
- ~403,000 individuals with cryptosporidiosis
- via contaminated drinking water
- ~illness-associated cost of US $96.2 million
- ~100 deaths (2)

**Second largest waterborne outbreak occurred in:**
- Östersund, Sweden in 2010
- ~27,000 individuals infected (3)

**Another waterborne outbreak:**
- Skellefteå, Sweden in 2011
- ~18,500 individuals (4)

INCREASE OF:

- Waterborne diseases
- Foodborne diseases
- Vectorborne diseases
Unlimited Mobility
MIGRANTS & REFUGEES
Transmission of *Cryptosporidium*

- Human - to - Human
- Animal - Human - Animal
- Water
- Food
- Air
Cryptosporidium Life Cycle

1. Thick-walled oocyst (sporulated) exits host
2. Contamination of water and food with oocysts.
3. Thick-walled oocyst ingested by host

Gastroenteritis

Food

Recreational water

Drinking water
Identify the contamination sources!
CRYPTOSPORIDIUM IN WATER & FOOD
Main causes of foodborne Cryptosporidium outbreaks usually associated with food service and catering industries:

- Cold pressed non-alcoholic apple cider
- Ozonated apple cider
- Milk
- Béarnaise sauce
- Raw meat, raw liver
- Chicken salad
- Pre-cut mixed salad leaves (bagged salad)
- Frisée salad
- Fruit juice
- Molluscs
- Sprouted seeds

Highly expected of being vehicles for transmission of Cryptosporidium: freshly produced, and when consumed with minimal preparation.

33 years review (Ahmed & Karanis, 2017, in press): *Cryptosporidium* detection in food material

**Food Material**

- Vegetables and Fruits
  - Elation
  - Simple Centrifugation
  - IMS
  - Molecular
    - PCR
    - FISH
    - LAMP
    - Aptamers

- Meat
  - Elution
  - 1M Glycine
  - Detergent based buffer
  - Concentration / Purification
  - Detection

- Shellfish
  - Homogenization / Tissue Aspiration
  - Elution
  - Concentration / Purification
  - Calcium chloride
  - Sucrose gradients
  - Lipid extraction

- Beverages
  - Apple Juice, Milk, Orange Juice, Ice cream
  - PET TBS

**Methods**

- Stomaching
- Orbital shaking
- Pulsification
- Kneading
- Sonication
- Rotating Drum
- Adhesive tape
- Pepsin digestion

**Buffers**

- HEPES pH 5.5
- Sodium bicarbonate pH 6
- 1M Glycine pH 5.5
- Bicine pH 5.5
- 1% Lauryl sulphate
- EB
- Tricine pH 5.4
- PBS
- Deionized water
- 1% Alconox
Cryptosporidium was ranked 5th out of 24 potentially foodborne parasites in terms of importance as a foodborne pathogen.
Cryptosporidium is among the 10 foodborne pathogens monitored by the FoodNet surveillance system in the United States
Cryptosporidium is particularly suited to foodborne transmission and is responsible for > 8 million cases of foodborne illness annually.
33 years review (Ahmed & Karanis, 2017): Documented outbreaks of cryptosporidiosis associated with different food materials 1984-2017

<table>
<thead>
<tr>
<th>Infected material</th>
<th>Food material</th>
<th>Causative species/subtype of <em>Cryptosporidium</em></th>
<th>Location of outbreak</th>
<th>No. of affected people</th>
<th>Year of outbreak occurrence</th>
<th>Reference/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sausage</td>
<td>NP</td>
<td>NP</td>
<td>UK (North Wales)</td>
<td>19</td>
<td>1984</td>
<td>Casemore et al., 1986</td>
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<tr>
<td>Milk</td>
<td>NP</td>
<td>Milk</td>
<td>Mexico</td>
<td>22</td>
<td>1984</td>
<td>Elsser et al., 1986</td>
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<tr>
<td>Kefir</td>
<td>NP</td>
<td>Kefir prepared in the nursery milk kitchen</td>
<td>Russia</td>
<td>13</td>
<td>1990</td>
<td>Romanova et al., 1992</td>
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<tr>
<td>Fresh pressed apple cider</td>
<td>C. parvum</td>
<td>France (central Maine)</td>
<td>160</td>
<td>1993</td>
<td>Millard et al., 1994</td>
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<tr>
<td>Chicken salad</td>
<td>C. parvum</td>
<td>US (Minnesota)</td>
<td>15</td>
<td>1995</td>
<td>CDC, 1996</td>
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<td>School Milk</td>
<td>NP</td>
<td>School Milk</td>
<td>UK</td>
<td>50</td>
<td>1995</td>
<td>Gelletlie et al., 1997</td>
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<td>Uncooked green onions (suspected)</td>
<td>C. parvum</td>
<td>US (Spokane)</td>
<td>54</td>
<td>1995</td>
<td>CDC, 1997</td>
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<td>Unpasteurized milk</td>
<td>NP</td>
<td>Unpasteurized milk</td>
<td>USA</td>
<td>144</td>
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<td>Blackburn et al., 2006</td>
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<td>Vojdani et al., 2008</td>
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<td>Yoshida et al., 2007</td>
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<td>Insulander et al., 2008</td>
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<td>Rimselene et al., 2011</td>
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<td>McKerr et al., 2015</td>
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<td>Åberg et al., 2015</td>
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Foodborne outbreaks of cryptosporidiosis have been increasingly reported, however most foodborne outbreaks are never recognized, and those that are recognized, frequently are poorly investigated - if at all, and often go unreported.
40 years - Overview (Efstratiou, Ongerth, Karanis 2017): Detection methods for *Crypto & Giardia* monitoring

<table>
<thead>
<tr>
<th>Microscopy Procedures</th>
<th>Conventional staining</th>
<th>Immunochemistry assay</th>
<th>Immunofluorescence assay</th>
<th>Microscopy tools</th>
<th>Others</th>
<th>Spectroscopy</th>
<th>PCR</th>
<th>Molecular Procedures</th>
</tr>
</thead>
</table>

Table 3
Overview of the literature relating to detection/identification methods used during Phase 3 of the Cryptosporidium and Giardia monitoring procedure. The table refers to the original research articles for further information.

<table>
<thead>
<tr>
<th>Detection methods for <em>Crypto &amp; Giardia</em> monitoring</th>
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<tr>
<td>Ajeagah et al., 2007</td>
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<td>Rodriguez-Hernandez et al., 1994</td>
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<td>Smith et al., 1989</td>
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<td>Smith et al., 1989</td>
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<td>Sauch, 1985, Ongerth and Stibbs, 1987</td>
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<td>Shaw et al., 1977, Holman et al., 1983</td>
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<td>Sauch, 1985, Payment et al., 1989</td>
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<td>deRegnier et al., 1989, Smith et al., 1991</td>
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<td>Mudanyali et al., 2010</td>
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<td>El Mallahi et al., 2013</td>
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<td>Houssin et al., 2010</td>
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<td>Sturbaum et al., 2001, Xiao et al., 2001, Nichols et al., 2003</td>
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<td>Guy et al., 2003, Foulds et al., 2002, Fontaine and Guillot, 2003, Yu et al., 2010, Stagg et al., 2013</td>
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<td>Karanis et al., 2013, Plutzer and Karanis, 2009, Plutzer et al., 2010, Koloeren et al., 2011</td>
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<td>Inomata et al., 2009</td>
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<td>Lee et al., 2010, Brinkman et al., 2013</td>
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<td>Baeminser et al., 2001</td>
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<td>Rochelle et al., 1997a,b, Johnson et al., 2012</td>
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<td>Veal et al., 2000, Vesey et al., 1993b</td>
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<td>Ferrari et al., 2000, Ferrari and Bergquist, 2007, Chung et al., 2004</td>
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<td>Lepetenteur et al., 2003, Reseure et al., 2011, Reseure et al., 2012</td>
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<td>Jin et al., 2009</td>
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<td>Vesey et al., 1994</td>
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<td>Reynolds et al., 1999, Rushton et al., 2000, deRoubin et al., 2002</td>
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<td>Lee et al., 2001, Call et al., 2001</td>
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<td>Siddons et al., 1992</td>
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<td>Taguchi et al., 2006</td>
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<td>Taguchi et al., 2007</td>
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<td>Kang et al., 2006</td>
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<td>Campbell and Mutharasen, 2008, Xu and Mutharasen, 2010</td>
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<td>Angus et al., 2012</td>
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<td>Kramer et al., 2007</td>
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<td>Thruppathiraja et al., 2011</td>
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<td>Beale et al., 2013</td>
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<td>Lacza et al., 2013</td>
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</table>
Detection of *Cryptosporidium* oocysts

- by staining
  - e.g. Giemsa
  - Eosine
  - modified Ziehl-Neelsen-Staining = mZNS
  - in stool smears

- Immunfluorescence = IFT
- Copro-Antigen-detection (ELISA)
- Molecular detection (PCR)
40 years review (Ahmed & Karanis, 2017, in press): Cryptosporidium detection in stool material
Cryptosporidium in stool (Light microscope)
Cryptosporidium in stool (Light microscope, IFT)
Cryptosporidium in stool (Light microscope, IFT)
Cryptosporidiosis - endoscopy

Exogen toxic duodenitis with severe erythema. *Cryptosporidium* infection leads to malabsorption syndrome and diarrhea

TREATMENT / THERAPY
There is no effective treatment for cryptosporidiosis in all populations and no vaccine is available

Only symptomatic therapy!
PREVENTION

★ Wash hands, clean toilets
★ Boil water
★ Food (particular vegetables) good washing + boiling
★ Know and prevent the potential infection sources + transmission routes

Note: Carriers of Cryptosporidium – oocysts are sources for fecal-oral transmission
WATER TREATMENT & PATHOGEN REMOVAL
Filtration

Cryptosporidium removal by multimedia filters

Giardia Cryptosporidium Cyclospora
Water treatment

1. Backwash water (very dirty)

2. ‘First filtrate’
   (low turbidity - drinking water quality)

Note: No disinfection!
Unexpected events: dangerous for drinking water preparation

Thessaloniki, Greece, May 2018
Flash Floods Hit Rhodes, Greece

18 October, 2013 by Richard Davies in Europe

The Greek island of Rhodes was hit by gale force winds and heavy rainfall yesterday, leading to flooding of several buildings and widespread storm damage.

The storm first struck the south eastern Aegean island on 17th October. Strong winds uprooted trees and damaged cars and buildings. Flooding was reported on many of the main roads around Rhodes town and the resort of Lindos on the southern coast of the island. However, the worst affected area has been the north east of the island.

Heavy downpours and gale-force winds brought down trees and caused floods on the Rhodes on Thursday, damaging cars, blocking traffic and flooding homes. The basements of many buildings have been flooded, but the storm passed quickly and flood waters have begun to recede.
Cryptosporidium: What are the Problems?

- Outbreaks
- Waterborne
- Foodborne
- Children, elderlies, immunosuppressed at risk
- No disinfection
- No therapy
- Swimming pools
- Tourism industries / Hotel business in danger
Outbreaks of a parasitic infection called Cryptosporidiosis, linked to swimming pools and water playgrounds, have at least doubled since 2014.

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
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<tbody>
<tr>
<td>2011</td>
<td>20</td>
</tr>
<tr>
<td>2012</td>
<td>16</td>
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<td>2013</td>
<td>13</td>
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<td>2014</td>
<td>16</td>
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<tr>
<td>2015</td>
<td>na¹</td>
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<tr>
<td>2016</td>
<td>32²</td>
</tr>
</tbody>
</table>

¹ - Numbers for 2015 have not yet been collected. ² - 2016 data are preliminary, based on reports from 24 states received by February.

Source: Centers for Disease Control and Prevention
Frank Pompa, USA TODAY
New trend: Hotel Holiday Claim Business!

Holiday Illness Claims
HELPING YOU RECOVER WHAT YOU DESERVE

Suffered illness on a package holiday in Lanzarote?

CALL US NOW
0800 01 54321
New trend: Hotel Holiday Claim Business!

Has your family holiday
BEEN RUINED BY SICKNESS
in Spain?

Your Holiday Claims are experts at helping groups of
holidaymakers claim the compensation they deserve.

To find out how much compensation
you could claim, call us now on...

0330 100 2851

Your Holiday Claims
New trend: Hotel Holiday Claim Business!

Potential hygiene and illness problems at the Holiday Village Kos in Greece reported by reviewers.

Source: Your Holiday Claim
Spanish hotel owners say Brits took them for £42m in fake food poisoning claims after plans emerge to ban all-inclusive packages

Britain has been dubbed the “fake sick man of Europe” as ‘no win - no fee’ firms are encouraging Brits to put in lucrative sickness claims.

Unscrupulous “no win - no fee” firms are behind the lucrative sickness claims.

Hoteliers are demanding a block on Brits going all-inclusive.
Versicherungsbetrug

Britische Touristen erschleichen sich 50 Millionen Euro

09.05.2017, 13:24 Uhr | t-online.de, az

Der Strand in Alcudia auf Mallorca ist ein beliebtes Reiseziel (Quelle: fossiphoto/imago)

"Durchfall-Masche" auf Mallorca

Hunderte Betrüger identifiziert

20.10.2017, 22:54 Uhr | dpa, pdi


Versicherungsbetrug: Immer mehr Briten kommen Mallorca-Hoteliers teuer zu stehen

50 Millionen Euro Schaden 2016: Der Hotellersverband prüft, wie er den Tricks der Touristen beikommen kann

06.03.2017 | 09:33


Dozens of Brits fall ill in Tenerife resort after claims of guests ‘DEFECATING’ in pool

DOZENS of Brits staying at a Tenerife resort have fallen ill after claiming there was poo in the swimming pool.

By JYOTI RAMBHAI

Dozens of Brits suffer from a gastro illness at a Tenerife Resort after claims of poo in the pool

Brits staying at the four-star Holiday Village Be Live resort have reported symptoms of vomiting and diarrhoea.

Holidaymakers suffering from the gastro illness have made claims of poor hygiene at the swimming pools.
Multiple illnesses reported at Lanzarote’s TUI Family Life Flamingo Beach resort

Suffered illness on a package holiday in Lanzarote?

CALL US NOW
0800 01 54321

New trend: Hotel Holiday Claim Business!
Suggestions and Conclusions

1. Multi-barrier system for water treatment
2. Simplify detection and diagnosis procedures
3. Standardized Surveillance System in all countries + international networking
4. Revision of guidelines/recommendations for swimming pool users + prevention of transmission of infectious diseases
4. Education / skills
5. Develop culture system in vitro
6. Drug + therapy development
7. Cooperate with the experts
Cryptosporidium is one of the most important pathogen causing diarrhea + diarrhea related death
Underreported / Underdiagnosed
No therapy yet / No vaccine
Transmission by water + food
Swimming pools: Tourism / hotel business in danger
Fecal – oral route of transmission
Cooperate with the experts!
Thank you for your attention!

Prof. Dr. Panagiotis Karanis
E-mail contact: panagiotis.karanis@uk-koeln.de
Korrelation zwischen Trübung und Parasitenvorkommen im Erstfiltrat eines Mehrschichtfilters

Trübung
Cryptosporidium

Parasitenvorkommen in 100 l

0,32
0,24
0,45
0,51
0,5
0,1
0

0
5
10
15
20

Minuten nach Filteranlauf
DISTRIBUTION AND REMOVAL OF GIARDIA AND CRYPTOSPORIDIUM IN WATER SUPPLIES IN GERMANY

Panagiotis Karanis*, Dirk Schoenen** and H. M. Seitz*

* Institute for Medical Parasitology, Sigmund-Freud-Straße 25, University of Bonn, D-53127 Bonn, Germany
** Institute of Hygiene, Sigmund-Freud-Straße 25, University of Bonn, D-53127 Bonn, Germany

ABSTRACT

This study has been conducted to estimate the distribution of Giardia and Cryptosporidium in German water supplies and the removal efficiency of surface water treatment plants for Giardia and Cryptosporidium by conventional treatment. Water samples from six surface water treatment plants in different parts of Germany were simultaneously examined for Giardia and Cryptosporidium. Investigations for both parasites were carried out in the period from July 1993 until December 1995. The results confirmed the occurrence of Giardia and Cryptosporidium in surface and raw water, in intermediate steps after treatment, in back wash water and in final treated water. Giardia cysts were detected in 57.8% of the samples and Cryptosporidium oocysts in 36.2% of the samples.
<table>
<thead>
<tr>
<th>investigation period</th>
<th>water source</th>
<th>positive samples for parasites/number of investigated samples in %</th>
<th>Giardia positive samples pos./inv.</th>
<th>Giardia cysts/100 l, average (max.)</th>
<th>Cryptosporidium positive samples pos./inv.</th>
<th>Cryptosporidiumooocysts/100 l, average (max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.07.95-05.12.95</td>
<td>RW</td>
<td>80/105</td>
<td>67/105</td>
<td>88.2 (1314)</td>
<td>49/105</td>
<td>116 (1081)</td>
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<td>IMTS</td>
<td>50/150</td>
<td>30/150</td>
<td>2.86 (19.2)</td>
<td>44/150</td>
<td>25.23 (66.6)</td>
</tr>
<tr>
<td></td>
<td>DW</td>
<td>18/47</td>
<td>7/47</td>
<td>3.77 (16.8)</td>
<td>14/47</td>
<td>3.24 (20.8)</td>
</tr>
</tbody>
</table>

RW = raw water, IMTS = intermediate treatment steps, DW = drinking water,