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Effects of anisakid nematodes *Anisakis simplex* (s.l.), *Pseudoterranova decipiens* (s.l.) and *Contracaecum osculatum* (s.l.) on fish and consumer health☆

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The anisakid nematodes *Anisakis simplex* (Rudolphi, 1809), *Pseudoterranova decipiens* (Krabbe, 1878) and *Contracaecum osculatum* (Rudolphi, 1802) occur as third-stage larvae in marine fish products and may infect consumers ingesting raw or under-cooked fish products. Clinical symptoms associated with the infection, termed anisakidosis, vary from irritation of the oesophagus and stomach, via nausea, vomiting and diarrhoea to severe epigastric and abdominal pain. Third-stage larvae of *A. simplex* are found in the body cavity, musculature and various organs, *P. decipiens* occur mainly in the fish musculature (fillet) and *C. osculatum* larvae reside predominantly in the liver, body cavity, mesenteries and pyloric caeca. Preventive measures, including mechanical removal of worms, heat treatment or freezing to kill worms, are needed in order to reduce the risk of human infections. The anisakid life cycle involves several hosts. *A. simplex* nematodes use cetaceans (whales) as final hosts whereas *P. decipiens* and *C. osculatum* have their adult stage in pinnipeds (seals). Eggs released by worms in these hosts pass with feces to seawater where free-living third-stage larvae hatch from the eggs. Various invertebrates – including euphausiids, copepods and amphipods – feed on these larvae, become infected and serve as intermediate hosts. A range of fish species may serve as transport hosts following ingestion of infected invertebrates and the final stage develops after two additional moults in the stomach of marine mammals which consumed infected fish. Control measures may be implemented to reduce infections of fish stocks and thereby risk of human infections.

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1. Introduction

Fishborne larval nematodes belonging to the family Anisakidae are widespread in fish populations worldwide (Nadler et al., 2005; Mattiucci and Nascetti, 2008; Karpiej et al., 2013). Representatives from the three anisakid genera Anisakis, Pseudoterranova and Contracaecum apply marine mammals as final host, invertebrates as intermediate host and fish as transport host (Køie and Fagerholm, 1995; Køie et al., 1995). The infective third stage larva in fish is able to infect consumers ingesting raw or undercooked fish products which may elicit severe clinical symptoms (Ishikura et al., 1993). The disease is termed anisakidosis referring to the causative agent belonging to the family anisakidae. In order to differentiate infections caused by worms from the different genera the term anisakiosis is used for infections by Anisakis spp. (Ishikura, 2003), the term pseudoterranovosis is applied for infections caused by Pseudoterranova spp. (Margolis, 1977) and finally infections due to Contracaecum spp. should accordingly be named contracaecosis. Each of the three genera is comprised of several sibling species (Mattiucci et al., 2014; Timi et al., 2014) but in this review the focus has been placed on Anisakis simplex (s.l.), Pseudoterranova decipiens (s.l.) and Contracaecum osculatum (s.l.).

2. Morphology and diagnosis

Macroscopic inspection of the anisakid larvae may reveal some minor differences between the parasites. A. simplex larvae are whitish to transparent (Fig. 1A), P. decipiens larvae are reddish to brownish (Fig. 1B) and C. osculatum larvae appear transparent via greyish to brownish (Fig. 1C). Specific diagnosis is not possible by macroscopic inspection or light microscopy but the three worm types can be differentiated by simple light microscopic examination of cephalic structures (booring tooth, excretory pore location), caudal end (tapering or rounded with mucron spine) and gastrointestinal elements (presence or not of intestinal caecum and ventricular appendix) (Fagerholm, 1982; Mattiucci et al., 1998; Quiazon et al., 2009). Specific diagnosis must rely on further molecular investigations. Allozyme markers are valuable for differentiating between A. simplex, P. decipiens and C. osculatum (Mattiucci et al., 1998; Mattiucci and Nascetti, 2007, 2008) but sequencing of genomic DNA encoding ribosomal RNA (18S, ITS1, 5.8S, ITS2, 28S) has proven useful for further species confirmation (Zhu et al., 2007; Jabbar et al., 2013; Mattiucci et al., 2014). The sequencing of the mitochondrial genome of A. simplex (Kim et al., 2006) allowed more specific diagnosis. The application of mitochondrial markers (cox1 and cox2) has shown to be valuable for further taxonomic resolution and differentiation of sibling species (Mohandas et al., 2014; Mattiucci et al., 2014; Liu et al., 2015).

3. Life cycle

A. simplex, with the vernacular name herring worm (or whale worm), obtain the adult stage in the stomach of cetaceans where copulation and oviposition take place (Fig. 2A). Eggs pass with feces to the marine environment where eggs hatch and...
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The anisakid third stage larvae are not strictly host specific and a wide range of marine teleost species may be found infected in most waters ranging from the Atlantic via the Mediterranean and the Pacific to the Antarctic area (Adroher et al., 1996; Mattiucci et al., 1998; McClelland and Martell, 2001). Third stage larvae of anisakid worms are mainly ingested by fish when they predate on the crustacean intermediate host or teleostean transport hosts. In the fish stomach larvae are activated and penetrate the stomach wall in order to seek residence in the peritoneal cavity, musculature or organs such as liver (Levsen and Lunestad, 2010; Mehrdana et al., 2014). Severe inflammatory reactions with tissue deformation, including marked cellular infiltration in the stomach wall and mucosa, of Atlantic cod has been associated with numerous penetrating A. simplex larvae (Levsen and Berland, 2012) and the term “stomach crater syndrome” was applied for this pathological reaction. Atlantic salmon and sea trout returning to rivers in Scotland, England and Wales were found infected with a high number of A. simplex larvae causing a “red vent syndrome”, characterized by haemorrhages and inflammation around the vent (Beck et al., 2008; Noguera et al., 2009). This species clearly provokes an inflammatory reaction in salmonids (Haarder et al., 2013) which first is seen as attraction of inflammatory cells and partly encapsulation of the worm (Fig. 3A). Likewise, P. decipiens become encapsulated by host cells in cod muscle tissue (Fig. 3B) and C. osculatum in cod’s livers (Fig. 3C and 3D) and it is believed that the mere presence of larvae in the tissues affect the normal function of these organs. A. simplex infections may be associated with the loss of condition of fish hosts, but in cases where the larvae are sequestered outside essential organs the effect may be less harmful. A. simplex liver infections in North Atlantic cod can be severe (Fig. 4A) but the effect on liver condition of the host may not be as devastating as expected from the worm load. Thus, a large part of the parasite’s infrapopulation is located in an encapsulated state on the surface of the organs which may be observed when worms are removed by peeling off the surface layer of cod’s livers. Anisakid nematode larvae such as P. decipiens and C. osculatum may affect the physiological state, health and survival of the host. Codworm infection of the fish muscle reduces swimming performance in smelt and eel which can lead to increased mortality in the wild.
Excretions from *P. decipiens* contain several pentanols and pentanons and it has been suggested that these compounds act as local anaesthetics in the cod muscle during worm penetration (Ackman and Gjelstad, 1975) and effects on muscle contractility may therefore be expected. Reduced swimming abilities of a fish host will ease predation by marine mammals, including seals, whereby this pathogenicity factor will optimize the life cycle of anisakids. Decreased body mass indices were recorded in Antarctic ice-fishes carrying high burdens of *C. osculatum* larvae which may indicate a parasite induced host effect (Santoro et al., 2013). In recent years Danish and Polish investigators (Buchmann and Kania, 2012; Mehrdana et al., 2014; Horbowy et al., 2016) have documented a marked increase of *P. decipiens* and *C. osculatum* infections of Baltic cod when compared to studies in the 1970s and 1980s (Möller, 1975; Thulin et al., 1989; Myjak et al., 1994) when seal abundance was low. The cod living in the Southern Baltic area may be infected by up to several hundred third stage larvae of *C. osculatum* (Haarder et al., 2014; Mehrdana et al., 2014; Nadolna and Podolska, 2014) which may challenge integrity and function of this key organ and affect growth and the nutritional state (Mehrdana et al., 2014; Zuo et al., 2016). Although the tissue disturbance by *C. osculatum* larvae is significant, even in a relatively well nourished cod liver (Fig. 4B), the impact is more prominent when liver size has decreased (Fig. 4C). Such a negative association was already claimed by Petrushevski and Shulman (1955) studying *C. osculatum* infection of Baltic cod in the 1940s and 1950s. A negative association between high parasite loads and the fish population size was noted by Eero et al., 2015 demonstrating reduced abundance of larger cod, concomitant with increasing worm occurrence, despite successful recruitment of young cod. This notion was further substantiated by Zuo et al. (2016) who showed that young Baltic cod with a body length below 30 cm were largely uninfected whereas cod larger than 30 cm harbored severe worm burdens. It is nevertheless difficult from field data to separate the potential

Fig. 3. A. Histological section of third stage larva of *Anisakis simplex* in infected liver tissue of Atlantic salmon, *Salmo salar*. A tissue reaction encapsulates the worm (scale bar 0.5 mm). B. Histological section of third stage larva of *Pseudoterranova decipiens* in the musculature of Baltic cod, *Gadus morhua*. Host cells encapsulate the larva (scale bar 1 mm). C. Histological section of third stage larva of *Contracaecum osculatum* invading the liver of a Baltic cod, *Gadus morhua*, with marked encapsulation by host cells (scale bar 1 mm). Scanning electron microscopy of a tube shaped encapsulated material (host cells from the liver of a Baltic cod) after removal of *Contracaecum osculatum* third stage larva (scale bar 2 mm).
parasite-related effects on condition and liver indices from more manageable impacts such as feed availability (Buchmann and Børresen, 1988). Therefore controlled laboratory trials must be performed in order to confirm this phenomenon.

5. Anisakids and fish product quality

Third stage larvae of the three anisakid species affect the fish product quality differently. Larvae of *A. simplex* reach 10–30 mm in total length, a width less than 1 mm, are whitish to transparent and are not easily detected by the naked eye when they reside deeply embedded in fish fillets. When occurring in high numbers in the body cavity or on the liver surface of cod it can be detected but many of the worms can be removed before industrial processing. This can be achieved simply by peeling off the liver capsule with associated worm larvae which significantly reduce the parasite burden. Larvae in the fillets need further efforts for removal and it should be noted that allergens from *A. simplex* still may be present in the products after removal or killing of intact worms (Audicana et al., 1997). *P. decipiens* larvae are longer and may reach a total length of 4–5 cm and a width of 1–2 mm. It is often reddish or brownish in color, and in thin fish fillets worms can be detected by the naked eye although some worms can be over-looked in thicker fillets. The industry should apply candling and mechanical trimming of fish products in order to remove worms from the fish fillet which may otherwise be discarded by consumers (McClelland, 2002; Levsen et al., 2005). Cod activates a strong cellular host reaction when *P. decipiens* larvae penetrate the musculature and the reaction immobilizes the larva in an encapsulated stage which protects the live worm but also adds to the disturbance of the fillet structure. *C. osculatum* third stage larva is semitransparent to greyish with a length of 2–3 cm and a width of approximately 1 mm or less. In Baltic cod livers only a part of the worm is visible as the main part of the parasite is embedded in the tissue under the liver surface. Cellular host reactions encapsulate the larva in a thick layer of host cells leaving the worm in an inactive stage which may last for at least three years. When occurring in high numbers in relatively small livers the organ is left useless for the industry. Surface peeling of the liver is not an option as the *C. osculatum* larvae do not attach to the liver epithelium but mechanical, chemical and enzymatic treatment of the entire liver may be a possible worm extraction method.

6. Zoonotic potential

Species within the three genera are also known to cause anisakidosis in humans upon accidental ingestion of live third stage larvae in unprocessed sea food. The main part of recorded anisakidosis cases are caused by *A. simplex* but also *P. decipiens* and *C. osculatum* are being recognized as responsible for severe gastrointestinal infections of man (Table 1). *A. simplex* is responsible for the majority of anisakiosis cases (Ishikura et al., 1993) but *A. pegreffii* has also shown its human-pathogenic potential.

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**Fig. 4.** A. North Atlantic cod (*Gadus morhua*) liver heavily infected with encapsulated third stage larvae of *Anisakis simplex* on the organ surface. B. Relatively well nourished liver of Baltic cod infected with third stage larvae of *Contracaecum osculatum* which are encapsulated inside the liver tissue. Only parts of the worms are visible on the surface. C. Small nutritionally deprived liver from a Baltic cod heavily infected with encapsulated third stage larvae of *Contracaecum osculatum.*
Table 1
Geographic distribution and genus of the causative pathogen in anisakidosis cases.

| Location    | Genus of worm causing anisakidosis | References                                      |
|-------------|-----------------------------------|------------------------------------------------|
|             | Anisakis  | Pseudoterranova | Contracaecum |                                   |
| Australia   | –          | –              | +            | Shamsi and Butcher, 2011           |
| Canada      | +          | –              | –            | Couture et al., 2003              |
| Chile       | –          | +              | –            | Mercado et al., 2001; Torres et al., 2007 |
| Croatia     | +          | –              | –            | Mladineo et al., 2014             |
| Denmark     | +          | –              | –            | Andreasen and Jörring, 1970       |
| France      | +          | –              | –            | Bourree et al., 1995              |
| Germany     | +          | –              | +            | Schaum and Müller, 1967; Møller and Schröder, 1987 |
| Holland     | +          | –              | –            | Van Thiel et al., 1960            |
| Iceland     | –          | +              | –            | Skirnission, 2006                 |
| Italy       | +          | –              | –            | Fumarola et al., 2009             |
| Japan       | +          | +              | +            | Kagei and Isogaki, 1992; Nagasawa, 2012 |
| Korea       | +          | +              | –            | Yu et al., 2001; Choi et al., 2009; Na et al., 2013 |
| Norway      | +          | –              | –            | Lin et al., 2014                   |
| Spain       | +          | –              | –            | Repiso et al., 2003; Puente et al., 2008; |
| South Africa| +          | –              | –            | Nieuwenhuizen et al., 2006         |
| Taiwan      | +          | –              | –            | Li et al., 2015                    |
| USA         | +          | –              | –            | Pinkus et al., 1975; Amin et al., 2000 |

Numerous investigations of clinical cases have documented the pathogenicity of the anisakid worm larvae in man which has called for further investigations using experimental animals in order to further characterize pathogenesis. Rats, rabbits, dogs and pigs may be applied for experimental infections and studies have shown that *A. simplex* (Desowitz, 1986), *P. decipiens* and *C. osculatum* (Strem et al., 2015) penetrate the mucosa of the gastrointestinal tract. This can elicit eosinophilic granulomatous formation corresponding to reactions reported to be associated with human infections. *A. simplex* larvae penetrating the stomach wall, the intestine or performing extra-intestinal migrations in humans (Testini et al., 2003), may elicit severe clinical symptoms including epigastric pain, vomiting, diarrhoea and nausea (Kagei and Isogaki, 1992; Nagasawa, 2012). Systemic reactions include eosinophilia and IgE titer increase (Perteguer et al., 2000; Valiñas et al., 2001) and the IgE titer measurements can be used for screening of infection rates in populations (Lin et al., 2014; Mladineo et al., 2014). *P. decipiens* larvae will only in rare cases perform severe extra-intestinal migrations in the human accidental host (Little and MacPhail, 1972; Amin et al., 2000) but the problems associated with ingestion of raw infected fish meat must be framed because symptoms associated with even light infections of non-penetrating worms include diarrhoea, gastric pain, tickling feelings, vomiting and coughing from the gastro-intestinal system (Little and Most, 1973; Pinel et al., 1996; Skirnisson, 2006). Relatively few human cases of *C. osculatum* larvae causing anisakidosis have been reported but it cannot be excluded that it is under-reported due to misidentification of the causative agent. Symptoms reported include gastro-intestinal pain, vomiting, diarrhoea and nausea. In one Australian case the worm was expelled spontaneously with bowel motion after 5–6 weeks disease history whereafter symptoms resided (Shamsi and Butcher, 2011). Two cases have been reported from Japan (Nagasawa, 2012) and one from the Baltic area in Europe (Schaum and Müller, 1967). The latter case involved both surgery and subsequent anthelmintic treatment in order to cure the patient.

### 7. Allergy induced by anisakid worms

The symptoms, clinical manifestations and immunological reactions in anisakidosis patients suggest that allergic reactions at least partly are involved in the disease development (Daschner and Pascual, 2005; Daschner et al., 2005; Choi et al., 2009). The mechanical impact of penetrating larvae on inner organs may cause some symptoms but increase of serum IgE titers, eosinophilia (systemic and local), skin symptoms, urticaria, airway obstruction and even anaphylactic reactions call for additional explanations (Caballero and Moneo, 2002; Nieuwenhuizen et al., 2006). A range of allergens have been isolated from *A. simplex* (Desowitz, 1986), *P. decipiens* and *C. osculatum* (Strøm et al., 2015) penetrate the mucosa of the gastro-intestinal tract. This can elicit eosinophilic granulomatous formation corresponding to reactions reported to be associated with human infections. *A. simplex* larvae penetrating the stomach wall, the intestine or performing extra-intestinal migrations in humans (Testini et al., 2003), may elicit severe clinical symptoms including epigastric pain, vomiting, diarrhoea and nausea (Kagei and Isogaki, 1992; Nagasawa, 2012). Systemic reactions include eosinophilia and IgE titer increase (Perteguer et al., 2000; Valiñas et al., 2001) and the IgE titer measurements can be used for screening of infection rates in populations (Lin et al., 2014; Mladineo et al., 2014). *P. decipiens* larvae will only in rare cases perform severe extra-intestinal migrations in the human accidental host (Little and MacPhail, 1972; Amin et al., 2000) but the problems associated with ingestion of raw infected fish meat must be framed because symptoms associated with even light infections of non-penetrating worms include diarrhoea, gastric pain, tickling feelings, vomiting and coughing from the gastro-intestinal system (Little and Most, 1973; Pinel et al., 1996; Skirnisson, 2006). Relatively few human cases of *C. osculatum* larvae causing anisakidosis have been reported but it cannot be excluded that it is under-reported due to misidentification of the causative agent. Symptoms reported include gastro-intestinal pain, vomiting, diarrhoea and nausea. In one Australian case the worm was expelled spontaneously with bowel motion after 5–6 weeks disease history whereafter symptoms resided (Shamsi and Butcher, 2011). Two cases have been reported from Japan (Nagasawa, 2012) and one from the Baltic area in Europe (Schaum and Müller, 1967). The latter case involved both surgery and subsequent anthelmintic treatment in order to cure the patient.

### 8. Geographic distribution of anisakidosis

Anisakidosis cases have primarily been recorded in countries where consumption of undercooked fish is common. Anisakiosis cases are commonly reported in Japan (Oshima, 1987; Suzuki et al., 2010) but infections are also known from Korea, Taiwan,
Europe (Denmark, Norway, Germany, Holland, France, Italy, Spain, Croatia), North America (USA and Canada) and South Africa (Table 1). Most pseudoterranovosis cases have been reported from Japan (Sawada et al., 1983; Ishikura et al., 1993), Korea (Koh et al., 1999; Yu et al., 2001; Na et al., 2013), Iceland (Skrínisson, 2006), North America (Hitchcock, 1950; Chitwood, 1975; Kliks, 1983), South America (Mercado et al., 2001; Torres et al., 2007) but changing eating habits in most countries (involving dishes such as ceviche, sushi, sashimi and corresponding preparations) may expose consumers globally to infection. Human infections due to C. osculatum were reported from Germany (Schaum and Müller, 1967), Australia (Shamsi and Butcher, 2011) and Japan (Nagasawa, 2012) but due to the widespread and global occurrence of the species in a range of teleost species attention should be given to new cases of contracaecosis.

9. Treatment

Infections with anisakid third stage larvae may in some cases progress without symptoms and patients may be cured spontaneously. In other cases severe symptoms due to larval penetration of host tissues are evident and patients have been hospitalized and subjected to medical examination. By use of ultrasonography (Ido et al., 1998), fiber-gastroscopic and endoscopic equipment (Matsumoto et al., 1992) some worms may be localized in situ whereby immediate removal of the pathogen can be conducted. In other cases surgery involving resection of the affected organ area has shown necessary for diagnosis and treatment (Bourree et al., 1995; Pampiglione et al., 2002; Repiso et al., 2003). Treatment with anthelmintics may be a way for elimination of the causative agents. Thus, application of albendazole has been advocated for Anisakis infections in humans (Moore et al., 2002; Pacios et al., 2005) and thiabendazole was used successfully against a human C. osculatum infection (Schaum and Müller, 1967) when symptoms re-occurred after surgical removal of some worm larvae. A series of screenings have suggested that also herbal drugs may affect survival of anisakids. Thus, monoterpenes (Hieron et al., 2004, 2006), geraniol, citronellal (Barros et al., 2009) and Matricaria chamomilla oils (Romero et al., 2012) have all shown in vitro effects but require clinical trials for confirmation.

10. Prevention by food processing

The risk of contracting anisakidosis following ingestion of raw or semi-raw seafood products (Gardiner, 1990; Couture et al., 2003; Puente et al., 2008) have been known since the 1950s when the first case of anisakiasis was detected in the Netherlands (Van Thiel et al., 1960). Later this zoonotic problem was recognized in other countries including Japan where eating habits including raw fish dishes increased risk of infection (Oshima, 1987; Ishikura et al., 1993). Research focused subsequently on how worms could be rendered inactive and non-infective. Heating to 60 °C for minutes and freezing to −20 °C for 24 h were methods which were shown to kill worm larvae and recommendations for pre-treatment of fish products were published (Wharton and Aalders, 2002). Freezing regulations were then implemented in Holland and since then in the EU (EFSA, 2010). It is also known that prolonged salting at high NaCl concentrations will kill anisakid larvae but a series of investigations have demonstrated that vinegar and salt used in marinating procedures do not readily inactivate worm larvae as this process may take weeks (Karl et al., 1994). Therefore the recommendations for marine food products as stated above should be followed. Quality assurance and worm content status of fish products should be performed regularly by several methods. Test of product samples can be conducted by full artificial digestion in a pepsin and hydrochloric acid solution at 37 °C or by mechanical compression of fish products in order to record the worm content status. Candling on light tables may be used by not all anisakid larvae may be recovered by this method (Levensen et al., 2005) although the methodology has been improved (Yang et al., 2013). A. simplex and P. decipiens can be detected due to fluorescence if the fish fillet is compressed, frozen and subsequently illuminated by UV-light (Karl and Leinemann, 1993) but this does not apply for C. osculatum in cod liver tissue (Zuo et al., 2016). Several immune-chemical and molecular techniques have been developed for detection of anisakids in fish products. Thus, enzyme linked immunosorbent analysis (ELISA) (Xu et al., 2010; Werner et al., 2011), restriction fragment length polymorphism following PCR (RFLP-PCR) (Espíñeira et al., 2010), realtime PCR (Lopez and Pardo, 2010; Herrero et al., 2011) may be used by the industry to secure the status of their products.

11. Prevention by management

If a parasite-free status of certain sea-food types can be documented the risk of anisakidosis should be significantly reduced. This may allow for the lifting of freezing regulations which are currently needed to inactivate anisakids. Aquacultured fish such as marine-cultured rainbow trout, Atlantic salmon, sea bass, sea bream and turbot, which have been raised in isolation from infected prey organisms and have been fed exclusively heat treated and parasite-free feed pellets, are eligible through adequate documentation for parasite-free status (EFSA, 2010). However, one of the reasons that the problem with worm infected fish products is increasing in certain geographic areas is associated with the increasing populations of marine mammals. Seal population sizes in Iceland were previously shown to be correlated with P. decipiens infections (Hauksson, 2002, 2011). Similar associations were shown in Norway (Jensen and Idås, 1992) and recently grey seal population in the eastern Baltic sea has expanded significantly to around 50,000 individuals since the year 2000 leading to increased infections of P. decipiens and C. osculatum (Buchmann and Kania, 2012; Mehrdana et al., 2014; Horbowy et al., 2016). It is a local and relatively stationary seal population living together with a local and stationary cod population. Thus, the local cod population has experienced a marked increase of P. decipiens and C. osculatum infection levels.
It may be debated whether regulation of marine mammal populations in specified areas should be implemented in order to reduce the effect on fish stock size and fish product quality. Such management efforts may conflict with the conservation of the final host populations which often have protected status. The effect of hunting (culling) efforts and contraceptive measures should be evaluated in this regard. The use of anthelmintics in mass treatment of final hosts, such as seals, in order to reduce the general infection pressure, may be viewed as a theoretical option, but this solution raises environmental concerns and may be difficult to perform on a large scale.

Conflict of interests

The authors declare that they have no conflict of interests.

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