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Review

Zebrafish: an animal model for research in veterinary medicine

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Abstract

The zebrafish (*Danio rerio*) has become known as an excellent model organism for studies of vertebrate biology, vertebrate genetics, embryonal development, diseases and drug screening. Nevertheless, there is still lack of detailed reports about usage of the zebrafish as a model in veterinary medicine. Comparing to other vertebrates, they can lay hundreds of eggs at weekly intervals, externally fertilized zebrafish embryos are accessible to observation and manipulation at all stages of their development, which makes possible to simplify the research techniques such as fate mapping, fluorescent tracer time-lapse lineage analysis and single cell transplantation. Although zebrafish are only 2.5 cm long, they are easy to maintain. Intraperitoneal and intracerebroventricular injections, blood sampling and measurement of food intake are possible to be carry out in adult zebrafish. *Danio rerio* is a useful animal model for neurobiology, developmental biology, drug research, virology, microbiology and genetics. A lot of diseases, for which the zebrafish is a perfect model organism, affect aquatic animals. For a part of them, like those caused by *Mycobacterium marinum* or *Pseudoloma neutrophila, Danio rerio* is a natural host, but the zebrafish is also susceptible to the most of fish diseases including Itch, Spring viraemia of carp and Infectious spleen and kidney necrosis. The zebrafish is commonly used in research of bacterial virulence. The zebrafish embryo allows for rapid, non-invasive and real time analysis of bacterial infections in a vertebrate host. Plenty of common pathogens can be examined using zebrafish model: *Streptococcus iniae, Vibrio anguillarum* or *Listeria monocytogenes.* The steps are taken to use the zebrafish also in fungal research, especially that dealing with *Candida albicans* and *Cryptococcus neoformans.* Although, the zebrafish is used commonly as an animal model to study diseases caused by external agents, it is also useful in studies of metabolic disorders including fatty liver disease and diabetes. The zebrafish is also a valuable tool as a model in behavioral studies connected with feeding, predator evasion, habituation and memory or lateralized control of behavior. The aim of the present article is to familiarize the reader with the possibilities of *Danio rerio* as an experimental model for veterinary medicine.

Key words: zebrafish, veterinary, bacteria, viruses, fungi, metabolic, diseases

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Fig. 1 Adult wild-type female zebrafish with normal body length, eyes and pigmentation (Santoriello et al. 2010).

Introduction

The zebrafish (*Danio rerio*) is a small, tropical, freshwater fish, native to rivers of South Asia (Fig. 1). It has become known as an excellent model organism for studies of vertebrate biology and particularly in studies of vertebrate genetics and embryonal development. The zebrafish is also a potential model for diseases and drug screening (Panula et al. 2006). Nevertheless, there is still lack of detailed reports about usage of the zebrafish as a model in veterinary medicine.

Adult zebrafish are small (approximately 2.5 cm long), have a short generation time (3-4 months), and they can lay hundreds of eggs at weekly intervals, which make them useful to perform various research. Moreover, transparent egg wall gives an opportunity to monitor the development of fish, and furthermore, use it for particular research. The housekeeping of zebrafish is much cheaper than rats or mice and requires less space, comparing to the rainbow trout, a model organism popular in aquaculture. Advantages of *Denio rerio* cause that it is a useful animal model for neurobiology (Haffter et al. 1996, Panula et al. 2006), developmental biology, drug research, virology (Phelan et al. 2005, Ludwig et al. 2011), microbiology (Swaim et al. 2006, Levraud et al. 2009) and genetics (Petrie-Hanson et al. 2009). Teleost fish species, such as the zebrafish, have a well-developed immune system, both innate and adaptive, which is similar to the mammalian immune system (Zarkadis et al. 2001). As a consequence, important genetic tools that enable to design experimental investigations using zebrafish have been developed (Wienholds et al. 2002). The transgenic animals and mutants are commonly used in laboratories worldwide (Haffter et al. 1996, Petrie-Hanson et al. 2009). Comparing to embryos of other vertebrates, externally fertilized zebrafish embryos are accessible to observation and manipulation at all stages of their development. Furthermore, there are plenty of research techniques, such as fate mapping, fluorescent tracer time-lapse lineage analysis and single cell transplantation, which improve the quality and diversity of zebrafish research. Unfortunately, the zebrafish is still poorly exploited as a laboratory model in studies on animal diseases, for which it could be a precious acquisition. Therefore, the aim of the present article is to familiarize the reader with the possibilities of *Danio rerio* as an experimental model for veterinary medicine.

Genetic diseases

Mutations may be tolerated in an organism without any noticeable effect, or they may show up with a specific phenotype, which gives information about the function of changed genes. This information is an important source for understanding biology, mechanisms of development, degeneration and diseases. The zebrafish has been proven to be a perfect instrument in the genetic analysis of spontaneous or induced mutations, which provides valuable information about the complexity of molecular processes in vertebrate biology (Ackermann et al. 2003). Tools of molecular biology, including gene sequencing, DNA microarrays and various types of PCRs, give the researchers an opportunity to study the zebrafish genome, especially fragments which are active during embryonic development and their malfunctions lead to diseases and congenital disorders.

Among the zebrafish mutants, *sox10* is one of the most known. Gene *sox10* in the mutant, also known as *colourless* (*cls*), shows a strong conservation of gene expression pattern and function (Dutton et al. 2009). This mutation responds both for depigmentation and agangliosis, mainly in the inner ear and enteric nervous system (Carney et al. 2006). *Sox 10* mutant may be used as an animal model to study several diseases, including the lethal white syndrome in foals, congenital megacolon in dogs and cats, or congenital deafness among domestic animals, which is often associated with the coat pattern. All these defects are results of failure in migration of the neural crest cells (NCCs) to the hindgut or inner ear during embryogenesis. The migration process is under the control of *sox 10* gene (Kelsh et al. 1996, 2002). During nervous system development sox proteins interact with each other, but the transcript factors, which these proteins interact with influencing the development, are still not fully

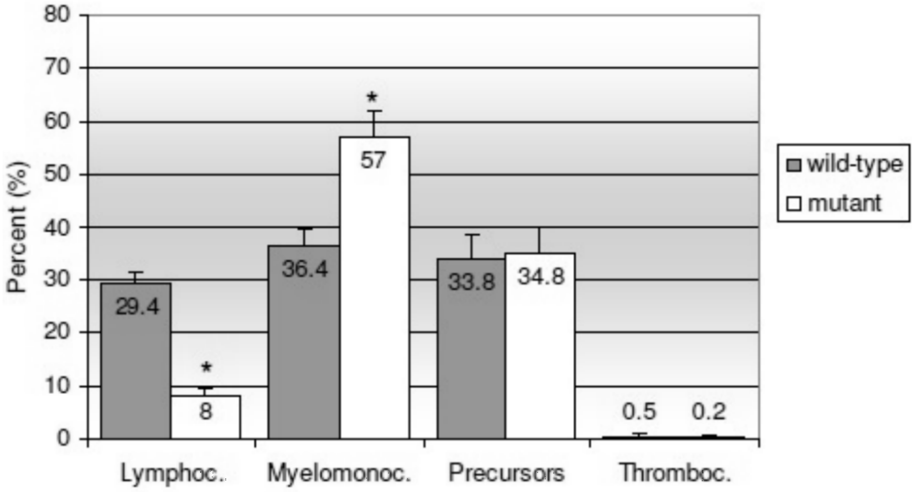

Fig. 2 The table shows count of peripheral blood smears and kidney hematopoietic tissue smears. Asterisks indicate important difference between wild-type and mutant blood cells within the specific populations. Average percentage + standard deviation from 10 replicates is shown (p≤ 0.05) (Petrie-Hanson et al. 2009).

identified. Even that *sox10* is required for multiple diverse cell types, it has been clearly demonstrated that this transcription factor is insufficient to explain the fate of neural cells (NC) during their differentiation (Wegner et al. 2005). In general, fate specification of NC requires both intrinsic factors and extrinsic signals for proper differentiation (Wegner et al. 2005). Identification of all of these factors will be necessary for a comprehensive understanding of the logic of NCCs fate specification. *Rag 1* is another interesting mutant that can be useful in the field of veterinary medicine. Comparing to the wild-type, *rag1* mutants have a significantly reduced population of non-specific cytotoxic cells (NCC) and natural killer (NK) cells and lack of functional T and B lymphocytes (Fig. 2). On the other hand, macrophage/monocyte population is similar both in mutant fish and wild-type, whereas in r*ag1* mutants neutrophil population is higher. These mutants can be a perfect model for a syndrome called severe combined immunodeficiency (SCID), a genetic disorder which is characterized by the immunodeficiency. Currently, *rag1* and *rag*2 mutant mice have been already used as animal models for SCID research. In mutant mice, comparing to the zebrafish, levels of macrophages, natural killer cells and neutrophils are standard, but there are neither T nor B lymphocytes (Petrie-Hanson et al. 2009). This situation leads to the conclusion that zebrafish could be a better model for this disease than mice, because of the general advantages as a laboratory animal and its higher similarity to the immunodeficiency presented in SCID disorder. Mutation of gene in the *rag1* mutant results in a block of immunological gene assembly and leads to immunodeficiency. The disease appears most often in Welsh Corgi's and bassets, and is sex-related (The Merck Veterinary Manual, 10th edition, 2010). *Rag1* mutant zebrafish can be an excellent model for studies on fish and innate immunology, and replace *rag 1* and *2* mutant mice in the field of mammalian immunology (Petrie-Hanson et al. 2009).

Morpholino, oligomers antisense to specific sequences in nucleic acids that is used to modify gene expression, is widely used among zebrafish researchers. It evokes knock down of targeted genes in the zebrafish which let us to take a new look at many diseases. Krabbe's disease pathogenesis is a lethal inherited neurodegenerative disorder characterized by the mutation in gene which codes beta galactosidase. It results in decreased, or no activity of this enzyme and accumulation of the neurotoxic metabolite, galactosylceramide in the central nervous system cells. Consequently, galactosylceramide is not going to be metabolized into ceramide, the resource for myelin. As an effect, affected dogs and cats have no myelin in the nervous system. Although the mostly affected breeds are Cairn Terriers and West Highland White Terriers (The Merck Veterinary Manual, 10th edition, 2010), the disease has been also reported once in Miniature Poodle (The Merck Veterinary Manual, 10th edition, 2010) and one dog of unknown breed (The Merck Veterinary Manual, 10th edition, 2010). Lysosomal galactosylceramidase (GALC) activity has been demonstrated in the brain of zebrafish, both adults and embryos. After knock-down by using antisense morpholino oligonucleotides, *galca*/*galcb* morphants were obtained, characterized by loss-of-function of GALC activity in the central nervous system of developing zebrafish embryos (Zizioli et al. 2014) and they can be used in Krabbe's disease studies. This research enables to take a new look into the pathogenesis of Krabbe's disease and indicates potential of the zebrafish in studying lysosomal neurodegenerative diseases.

Bacterial diseases

Animal models are commonly used in studies of bacterial virulence. The zebrafish embryo allows for rapid, non-invasive and actual analysis of bacterial infections in a vertebrate host (Neely et al. 2002). To use the zebrafish as a model for bacterial diseases, a pathogen which is virulent both for fish and higher vertebrates is required.

Bacterium that meets all of these criteria is the Gram-positive organism *Streptococcus iniae* (Neely et al. 2002). It is a natural and important pathogen of fish that causes disease in various fish from all over the world. It affects both saltwater and freshwater species including tilapia, red drum, hybrid striped bass and rainbow trout. *Streptococcus iniae* was originally isolated in 1970s from subcutaneous abscesses of Amazon freshwater dolphin. Since then, *S. iniae* has been defined as a leading fish pathogen, of which mortality varies from 30% to 50% in fishponds affected (Neely et al. 2002). The signs and symptoms are various depending on the affected species. The bacterium attacks fish through wounds and colonize skin as well as the central nervous system. It may result in meningoencephalitis, lethargy, necrotizing dermatitis and skin erosion. Infection of the zebrafish has the same signs as in the natural hosts, and therefore it may be used in research of pathogenesis and virulence of *S. iniae,* which is obviously closely related to other bacteria that belong to the *Streptococcus* genus.

Another pathogen, for which *Danio rerio* is naturally susceptible, is *Mycobacterium marinum*, a close genetic relative of the causative agent of human, avian and bovine tuberculosis. The disease is characteristic among aquatic flora and has been detected in more than 20 species of marine and freshwater fish, frogs, and in a captive sea turtle (Ucko et al. 2005). Rapid development of fish farming and the ornamental fish industry leads to a worldwide increasing in the number of *M. marinum* infections in fish (Ucko et al. 2005). The main symptoms include loss of scales and color, lesions on the body, wasting and skeletal deformities such as curved spines. A zebrafish embryo model of infection has been used to characterize the earliest signs of the disease, when macrophages aggregate and make characteristic for tuberculosis granuloma forms. Research on *M. marinum* and its virulence in zebrafish extends the usage of the adult *D. rerio* to study a variety aspects of tuberculosis: from immunology to pathogenesis and drug tolerance (Swaim et al. 2006).

Zebrafish embryos are also model host for the real time analysis of *Salmonella typhimurium* infections. *Salmonella typhimurium* and *S. enteritidis* are the most frequent pathogens that affect people and animals, however, the basics of their pathogenicity remain still unclear. Strains from these groups produce severe diseases including typhoid in very young animals. The disease occurs when newborns receive insufficient protective antibody from their mothers, which results in decreased antibodies production and higher susceptibility to infection. The bacterium can also affect an adult organism as a result of old age, disease or pregnancy. The symptoms are various; in young calves, piglets, lambs, and foals either the enteritis or septicemic form might develop. In adult cattle, sheep, and horses acute enteritis appears most often. Chronic form of the disease may develop in growing pigs and occasionally in cattle, and can cause abortion in the pregnant animals. Salmonellosis is found infrequently in dogs and cats and is characterized by acute diarrhea, with or without septicemia. For studies on zebrafish model, the lipopolysaccharide (LPS) mutants of *S. typhimurium* have been used. The pathogen is attenuated and due to that it is non-pathogenic for the zebrafish embryos. Nevertheless, injection of LPS mutants into the yolk of the embryo results in uncontrolled bacterial proliferation, whereas heat-killed, wild-type bacteria are completely lysed within few minutes after the injection. These evidences show that blood of the zebrafish embryo can be successfully used for further studies of salmonellosis (van der Sar et al. 2003).

Another bacterium that is potentially dangerous for animals and can be examined with the zebrafish help is *Escherichia coli*. This pathogen is both commensal and pathogenic in a variety of animal hosts. It has the possibility to persist inside the gut among other microorganisms without any overt consequences. However, outside the intestinal tract, *E. coli* pathotypes can cause a wide range of diseases (Wiles et al. 2009). Enteric colibacillosis is a common disease of both nursing and weanling pigs and production birds raised for human consumption. The signs are varied including dehydration, peritonitis, meningitis, urinary tract infection and septicemia. Pathological distention of the small and big intestine with yellowish mucus can be observed. Because of its several advantages, the zebrafish has been developed as a model for *Escherichia coli* infection. It allows to investigate high phenotypic diversity of extraintestinal pathogenic strains of *E. coli* (ExPEC) that produces different, but significant for the disease toxins (Wiles et al. 2009). Previous attempts with more traditional animal hosts finished with a failure. That stabilizes the position of the zebrafish among other animal models and encourages to continue the research.

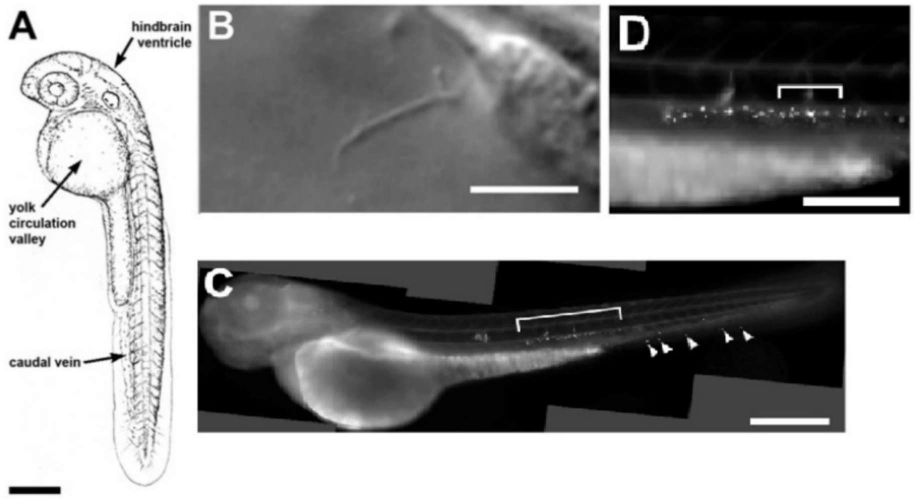

Fig. 3 A schematic view of 36 hours post fertilization zebrafish embryo (A). Phagocytes containing leptospires after 2 hours post intravenous infection (B). Whole embryo infected intravenously with SYTO 38-stained leptospira. Arrows show fluorescent leptospires around the ventral tail (C) and dorsal aorta (D) (Davis et al. 2009).

The zebrafish is used also as a model for leptospirosis infection. Although much has been learned about the biology and transmission of *Leptospira* species, the mechanisms of their pathogenesis and host colonization remain constantly unknown (Davis et al. 2009). The disease attacks all mammals and has a broad range of clinical effects from mild, subclinical infection to multiple-organ failure and death. Leptospires colonize the tubules of the kidney in a host organism for a short period of time and are shed in the urine for a few days to several weeks. They can also occur in the genital tract of permanently infected animals from months up to years after initial infection. Sometimes leptospires appear even in eyes of infected animals, leading to uveitis which is well known in veterinary medicine as periodic ophthalmia or moon blindness in horses (Rathinam 2005, Verma et al. 2013), a serious problem among veterinarians specialized in large animals. A variety of animal models of leptospirosis have been tested, each with its own advantages and disadvantages, but the zebrafish embryo allows to track *in vivo* interactions between the host and pathogen (Fig. 3) using microscopy techniques and fluorescently labeled bacteria. In the embryo, the infected cells are found in hematopoietic tissue lateral to the dorsal aorta (Davis et al. 2009). From this area leptospires can attack different organs during dissemination, while migrating from the caudal hematopoietic tissue. This observation is crucial for the understanding of pathogenesis of various diseases including *Leptospirosis.*

Vibrio anguillarum, also known as *Listonella anguillarum*, causes vibriosis, a deadly haemorrhagic septicaemic disease affecting various marine and fresh/brackish water fish, bivalves and crustaceans, mostly in late summer (O'Toole et al. 2004). The disease has been reported in the salmon, the bream, the eel, the mullet, the catfish and the tilapia. It is transmitted horizontally through direct contact. *Vibrio anguillarum* is responsible for severe economic losses worldwide, especially in the aquaculture (O'Toole et al. 2004). The main signs of the diseases are hemorrhages that cover the skin and body of infected fish. In young fish splenomegaly can be also observed (Frans et al. 2011). The zebrafish has been used to visualize the infection by GFP-labelled *Vibrio anguillarum*. The infection can be visualized in the whole fish using microscopy techniques. Furthermore, the each life stage of single bacterium can be also observed. The zebrafish infection model reveals that the intestine and skin are the main targets of *V.anguillarum* and suggests the presence of chemotaxis in a host body during the infection (O'Toole et al. 2004).

Listeriosis is an infectious but not contagious disease caused by the bacterium *Listeria monocytogenes*, which occurs commonly among domestic animals, wild animals, poultry and other birds. Infected animals suffer from encephalitis and gastro-intestinal septicemia, and pregnant animals may abort. In sheep, the disease is also called the „circling disease", with the signs concerning the nervous system, especially lateral deviation of the neck and head can appear. Although *L. monocytogenes* is known to contaminate seafood including fish products, it is not clear if fish are susceptible and actually can be infected by *Listeria spp*. Luckily, zebrafish embryos are susceptible to i.v. *Listeria monocytogenes* infection. Bacteria establish a systemic infection at 28°C and multiply in the host, resulting in death in about 3 days. The zebrafish larva represents a new host for the analysis of *Listeria monocytogenes* infection. Interactions between bacteria and host phagocytes can be imaged at high resolution *in vivo* from the earliest stages, and this model should be useful for the understanding of many events that until now could only be inferred from *in vitro* analysis (Levraud et al. 2009).

Staphylococcus is a gram-positive, coccoid pathogen that can affect a wide range of animals, especially avian species including poultry. *Staphylococcus aureus* occurs commonly among *Staphylococcus* species, but *S. hyicus* has also been reported as the causative agent of osteomyelitis in turkeys (Huff et al. 2000). The disease signs are varied, including gangrenous dermatitis, abscesses, arthritis, synovitis, osteomyelitis, endocarditis and septicemia which may lead to death. Economic losses may result from decreased weight, decreased egg production, lameness, mortality, and condemnation at slaughter. Although not a natural pathogen of the zebrafish, both embryos (Prajsnar et al. 2008) and adults show clear acute symptoms when infected with this gram-positive pathogen. Research of the embryos infected with fluorescent strain of *S. aureus* has been also done. After 2 days post injection (dpi) into the zebrafish bloodstream, the bacteria are visible in the embryos' leucocytes. The zebrafish embryo model investigations let us to discover the nature and relevance of the intracellular phase in the life cycle of this pathogen and give an opportunity to reveal non-specific host-pathogen interactions (Prajsnar et al. 2008).

As we can see the zebrafish becomes a popular animal model for bacterial diseases and it can be successfully used in the veterinary medicine. Not only fish bacteria can be investigated but also pathogens which are serious problems among domestic animals.

Viral diseases

The zebrafish is a perfect model to study viral diseases due to the fact that it is a natural host for many of these pathogens.

Iridoviruses are agents that cause severe diseases and provide economic losses among farmed fish, ornamental fish and wild fish around the world. Infectious spleen and kidney necrosis virus (ISKNV) is a member of this family and causes severe systemic diseases with high morbidity and mortality to more than 50 fish species: from Gourami, popular aquarium fish to mandarin fish *Siniperca chuatsi,* an important cultured freshwater fish in southern China. The signs and symptoms include anorexia, abnormal swimming, light body pigmentation and pale gills. Moreover, petechial hemorrhages in the operculum, mandible, eye, orbit, fin base and abdomen are shown in the infected animals. Swollen kidneys and spleen are pathognomonic signs of ISKNV infection (Tanaka et al. 2014). Adult zebrafish injected with ISKNV begin to reveal severe clinical symptoms at the 3rd day post infection. Furthermore, adult female zebrafish infected with ISKVN filtrate obtained from infected mandarin fish have been shown to reveal mortality of 65%. High susceptibility and presence of the characteristic pathological changes in zebrafish, confirm its reliability as a model for the research of ISKNV virulence in the host, which can be used to study the prevention and cure of ISKNV infection. Moreover, the genomes of both virus and zebrafish have been sequenced, giving an opportunity to explore the immune system of the fish as well as the functional genes of ISKNV (Xu et al. 2008).

Snakehead rhabdovirus (SHRV) belongs to *Rhabdoviridae* family, genus *Novirhabdovirus*. It affects various species of warm-water wild and pond-cultured fish in Southeast Asia, including snakehead species from which it is named. The clinical signs include high mortality with subepidermal petechial hemorrhaging and lethargy of infected fish. Experimental infection both of young and adult zebrafish results in the induction of characteristic symptoms and pathological sings. The pathological changes due to SHRV infection, by injection in adult fish, appear in the abdominal cavity at the site of the virus application. On the other hand, infection by immersion may be a more natural route of infection and allows the virus to spread throughout the fish body (Phelan et al. 2005). It is quite interesting that *Snakehead rhabdovirus* was the first virus which has been used to describe experimental infection of the zebrafish embryo with a viral pathogen. Thereby, the zebrafish has become an important infection model for future experiments using targeted gene disruption and genetic screening.

Another experimental viral infection of the zebrafish has been performed using IHNV virus. Infectious hematopoietic necrosis virus (IHNV) is RNA virus that is also a member of the *Rhabdoviridae* family from the genus *Novirhabdovirus*. It causes the disease known as infectious hematopoietic necrosis in salmonid fish, such as the trout and salmon. IHNV appears most often on the Pacific Coast of Canada and the USA, however, it has also been found in Europe and Japan. Clinical signs include abdominal distension, bulging of the eyes, skin darkening, abnormal behavior, anemia and fading of gills. Infected fish show hemorrhages in the mouth and behind the head, on the pectoral fins, in the anus area and in the yolk sac. Necrosis is a typical sign that occurs in the kidneys, spleen and rarely in the liver. Mortality is very high among young fish. Infection of zebrafish provides prominent clinical signs within three to four days. Investigations involving transgenic larvae of the zebrafish with fluorescent endothelium allow to detect infected cells in the whole organism and have revealed that main targets are blood vessels, that leads to typical clinical picture of the disease. Such studies have the potential to help designing more targeted, safer treatment regimens for viral diseases (Ludwig et al. 2011).

Spring viraemia of carp virus causes disease called Spring viraemia of carp or Swim bladder inflammation. Currently, it is listed as a species of the genus *Vesiculovirus* of the *Rhabdoviridae* family. The virus infects a wide variety of fish species including silver carp, grass carp, crucian carp, and bighead carp. It is considered to be a major pathogen to the native fish populations, especially farmed fish including ornamental koi and common carp. Typical signs and symptoms are viraemia and pathological lesions in kidneys, liver, spleen, heart and gastrointestinal tract. The other fish species, which are not a natural hosts of the virus, including the zebrafish have been experimentally infected. Signs and gross pathologic changes associated with SVCV infection of zebrafish include: partial to complete anorexia, listlessness, focal to multifocal epidermal petechial to ecchymotic hemorrhages and death. This indicates that zebrafish are susceptible to SVCV infection under conditions that mimic a natural route of exposure and gives a unique opportunity of using zebrafish wild-type strains and mutants to study the genetic basis of viral pathogenesis and host response in fish (Sanders et al. 2003).

Nervous necrosis virus (NNV) is an important fish pathogen, classified to the virus family *Nodaviridae,* that attacks the nervous system, mainly brain and retina. It occurs in teleost fish and has been reported in more than 40 species. Signs comprise abnormal behavior including lethargy, anorexia, spiral swimming and change in pigmentation. Microscopical lesions include necrosis and the presence of round empty spaces called vacuoles, located in the brain, retina and spinal cord. In the experimentally infected fish, brain, eye, heart, liver and gut have been tested positive for NNV presence. During histopathological examination, vacuolated cells can be observed in the brain tissue from 4 days post-infection onwards. The zebrafish infection model will provide a convenient system to study NNV pathogenesis and its potential treatment. This model can be further used for virus pathogenesis studies, such as apoptosis regulation, signal transduction and immunological response (Lu et al. 2008).

Infectious pancreatic necrosis (IPN) is a viral disease of salmonid fish, caused by infectious pancreatic necrosis virus (IPNV), a member of the *Birnaviridae* family. The disease is common among salmonids, such as trout and salmon, younger than six months, although older fish could also show signs of infection. It is another virus that can be used to infect zebrafish experimentally. Clinical signs contain abdominal swelling, anorexia, abnormal swimming, darkening of the skin and trailing of the feces from the vent. Necrosis of the pancreas and thick mucus in the intestines are often found. The ovarian transmission of virus, from adults to offspring, has been of great concern for many years. The zebrafish expands our knowledge about transmission and virulence of IPNV. Experimental injections of virus into the adult fish have revealed that the males appear to play no role in the transmission between parents and offspring. In addition, the virus is maintained in young zebrafish for a period of at least five months (Seeley et al. 1977).

As a conclusion, the zebrafish is a precious tool in viral researches that are important to veterinary surgeons. Zebrafish is a perfect animal model, because of its dual role: a natural host of pathogens and perfect laboratory organism. Although in the veterinary sciences the zebrafish has been used mostly to investigate fish viral diseases, the steps are taken to introduce it to studies of other species; diseases. Because of its advantages, *Danio rerio* should be used more widely in the veterinary research.

Parasitic diseases

The zebrafish remains a perfect system for analysis of microsporidia development, mainly because of its transparency and availability of visualize the infection with microscopy staining techniques. About 100 types of microsporidia are able to infect fish, including agriculturally relevant species, such as salmon and rainbow smelt (Troemel et al. 2011). Microsporidia are horizontally transmitted, spore-forming organisms that are extremely resistant and considered as non-treatable. *Pseudoloma neutrophila* is one that occurs most often in fish, including *Danio rerio* (Remsay et al. 2009). It affects the central nervous system, somatic muscle and is associated with emaciation, spinal deformity and morbidity (Kent et al. 2003). The spores create host-parasite complexes called xenomas (Lom et al. 2005), that multiple in the hindbrain, spinal cord, nerve roots, and sometimes within the skeletal muscles. The disease has often subclinical course and reveals its persistence when fish is under prolonged stress, resulting in immune suppression and increased susceptibility to infectious diseases. The infection has impact on growth, reproduction and may lead to higher mortality and decreased survival. Because the zebrafish is a natural host of *Pseudoloma neutrophila*, it has become a perfect model to study this pathogen. The zebrafish possesses advantages over other hosts like mammals and insects by making it possible to track the kinetics of the parasite development inside its transparent and hardy body (Troemel et al. 2011).

Ciliated protozoa are the most common external parasites of fish, which can be motile, attached or found within the epithelium. The most common pathogen that belongs to ciliates is *Ichthyophthirius multifiliis.* Infection caused by this pathogen is called „itch" or „white spot disease". It is transmitted horizontally and requires the presence of host to survive in the environment. The parasite attacks epithelial cells of gills, skin or fins and creates small wounds and white spots in the place of parasite encystation. The fish remain to be lethargic, covered with visible spots, the mortality can increase and be intensive. The zebrafish is a natural host species for *Ichthyophthirius multifiliis* and therefore remains to be an ideal model organism for research on this parasite. Moreover, the genome of *I. multifiliis* has been sequenced, which is a helpful tool for studies on this species. It could allow to reconstruct the development and metabolism of itch and compare it with the host's metabolic pathways, that would reveal potential targets for combating white spot disease (Coyne et al. 2011).

Parasitic diseases are often diagnosed problems among wild and domestic fish, especially itch, which is nightmare among private breeders and fanciers. Comparing to another model organism, catfish (*Ictalurus punctatus*), experimentally infected zebrafish presented lighter gill infection than catfish. Moreover, parasites collected from zebrafish demonstrated lower reproductive potential than parasites collected from catfish. Host mortality was also lower among the zebrafish, than catfish infected with itch (Cherry 2003). Due to that, a new experimental treatment methods can be carried out, because of natural susceptibility of the zebrafish to this pathogen combined with its excellent properties as a laboratory animal.

Fungal diseases

The zebrafish is used as a vertebrate host system to study the interface between fungal pathogen and the immune system (Harwood et al. 2014). *Candida albicans* occurs commonly in a variety of animals in every part of the world. It is a commensal organism that inhabits the nasopharynx, gastrointestinal tract and external genitalia in animals. Weakness of the immune system caused by stress, old age or pregnancy, can result in the clinical course of the disease. Clinical signs include watery diarrhea, anorexia and dehydration, with gradual progression to prostration and death. Economic losses concern reduced feed intake and growth rate in poultry and swine flocks. The zebrafish, due to its transparency, gives an opportunity to monitor the development and track infection using fluorescent imaging techniques. There are two forms of the pathogen cells, the hyphal and the yeast form, that are distinguished by their virulence (Harwood CG et al. 2014). Observations show that the non-hyphal mutant is less virulent in zebrafish larva (Harwood CG et al. 2014). Subsequently, the fungi switch to the more virulent hyphal form and proliferate in individuals that are more susceptible to the infection, whereas they revert to the yeast form in the most resistant embryos. Macrophages apparently have an enhanced ability to control the infection in *in vivo* environment (Torraca et al. 2014). Despite certain limitations of zebrafish embryos, they are incredibly useful and helpful organisms for generating testable hypotheses (Harwood et al. 2014).

Currently, the efforts are taken to use *Danio rerio* as a model for other fungal diseases. The most promising species is *Cryptoccocus sp.* (Sabiiti et al. 2012). An infection model of the zebrafish and *C. albicans* gives hope to the idea, that a disease model for *Cryptococcus* species could soon be developed. This confirms that the zebrafish could be a valuable tool in diagnostic and treatment of fungal diseases in animals that are common problems for veterinary surgeons.

Metabolic diseases

Although the zebrafish is used commonly as an animal model to study diseases caused by external agents, it is also useful in studies on metabolic disorders. It is possible due to the fact that *Danio rerio* possesses all organs required for metabolic controls, from the hypothalamus, through to the pancreas and insulin-sensitive tissues (Seth et al. 2013).

The zebrafish is used as a model for fatty liver disease. It is a metabolic disorder that occurs mostly in high milking cattle, when nonesterified fatty acid (NEFA) concentrations in blood are increased. This is the result of negative energetic balance and deficiency of energy in the organism. In the liver NEFA can be oxidized or esterified. The products of esterification are called triglycerides, and they can be either exported or stored. Because of the higher rate of NEFA in the organism, triglycerides accumulate, which leads to lower level of glucose and insulin in the blood. That results in blockage of the metabolic pathways of burning fats, which causes fatty liver disease and production of ketones. Fatty liver is associated with low milk production, increased clinical mastitis, and poor reproductive performance. Other metabolic disorders, that are associated with the disease, include reduced gluconeogenesis, ureagenesis, hormone clearance and hormone responsiveness. Further consequences, that may occur, are hypoglycemia, hyperammonemia and altered endocrine profiles. In the zebrafish, the formation of the liver primordium and the differentiation of hepatocytes and cholangiocytes can be observed after 48 hours post fertilization (hpf) (Chu et al. 2009). Several proteins are responsible for liver metabolism, including *uncoupling protein 2*, *phosphoenolpyruvate carboxykinase (pck1)* and *carnitine palmitoyltransferase 1A* (Gut et al. 2013). Staining with Oil Red O allows to detect adipocytes close to the gut and pancreas of zebrafish (Sadler et al. 2005). Intestinal lipids can also be observed *in vivo* using fluorescently labelled fatty acid analogues (Farber et al. 2001). The knowledge about genes that are involved in fats metabolism allows to knock them down using morpholino technique or produce mutant fish. For example, the *ducttrip* line of the zebrafish has mutation in *s-adenosylhomocysteine hydrolase* (*ahcy*) related gene. It is an enzyme that produces methyl donor for numerous biological processes (Seth et al. 2013). Mutant fish suffers from liver degeneration and hepatic steatosis (Matthews et al. 2009). This zebrafish mutant could be a precious model to study fatty liver disease and its new therapeutic options.

Danio rerio is used commonly to study pancreas development and diabetes. It is a chronic metabolism disorder due to insulin deficiency. The disease occurs in adult dogs, mostly females, and it is often connected with certain breeds such as Miniature Poodles, Dachshunds, Schnauzers, Cairn Terriers and Beagles. Adult, obese male cats are also included in the risk group but among this species there is no breed predilection. Common signs in dogs are polydipsia, polyuria, polyphagia with weight loss, bilateral cataracts and weakness. In cats, hepatic lipidosis may occur, which can lead to fatty liver disease. Furthermore, hepatomegaly can appear both in cats and dogs. The pancreas in the zebrafish contains exocrine and endocrine parts, similar to those found in mammals. Several genes have been shown to influence the development of β-cells in the zebrafish (Kinkel et al. 2009) and again this information has been used to produce lines of transgenic fish, useful in diabetes research. Beta-cells can be visualized in *Danio rerio* line, where the fluorescent reporter is placed under the control of the insulin promoter (Seth et al. 2013). The cells can be ablated by the addition of a prodrug, *metronidazole* (Mtz). *Nitroreductase* (NTR) catalyzes the reduction of the prodrug Mtz into cytotoxic product which leads to cell death (Seth et al. 2013). However, withdrawal of Mtz results in a restitution of the cells within 35 hours (Pisharath 2007). There is also another transgenic zebrafish line in which reporter expression is under the control of the *phosphoenolpyruvate carboxykinase (pck1)* promoter [*Tg(pck1:Luc2)*] (Gut et al. 2013). Pck1 is a hepatic enzyme, which limits gluconeogenesis. These transgenic lines allow to understand better regulation of diabetes and may be useful in the treatment of this deregulation.

Although atherosclerosis is a principal cause of death and morbidity in humans, it also occurs in pet birds and herbivores. The disease is common in old psittacine birds and the exception is African Grey parrot. In this species, even very young animals may suffer from the atherosclerosis. The right aortic arch may be enlarged with associated lipemia and marked elevations in blood cholesterol and triglycerides concentrations can be determined. The disease has been also reported in domestic and wild ruminants such as cattle, deer, goats and caribou, either old and young (Wiggers et al. 1971). In these species it is connected strictly with incorrect feeding and maintenance of animals. Lesions include thickening of vessels due to deposition of calcium, leading to degeneration of the tissue and large plaques visible during post-mortem examination. In zebrafish, a high cholesterol diet (HCD) can induce the same disorder and thus monitoring of its pathophysiology via fluorescent reporters and live imaging is possible. Here, again transgenic lines of the zebrafish are used. The zebrafish transgenic line *fli1:EGFP* with endothelial cells marked with green fluorescent protein, allows to visualize lipid accumulation in the vascular wall (Stoletov et al. 2009). Another transgenic zebrafish line *lyz:Ds Re* is used to study macrophage infiltration into adipose depots (Seth et al. 2013). This animal model also allows to monitor early pathogenic process of obesity (Bastard et al. 2005).

Conclusion

Several animal models have been developed to study various diseases important for veterinary medicine. Each has its specific advantages and is used for particular types of disorders. Although the zebrafish is not a mammal, it is attractive for a large group of scientists from all over the world. The first zebrafish publication was released in 1955, and only during the 2014 the number of the publications reached 2,397 positions.

Accordingly, *Danio rerio* has been used as a laboratory animal for studying viral, bacterial, genetic, fungal, parasitic and even metabolic diseases. Moreover, the zebrafish is also used widely as a model to investigate neurodegenerative diseases and their treatment (Panula et al. 2006, Podlasz et al. 2012), e.g. cognitive dysfunction syndrome in dogs (Landsberg 2005), which could be useful in veterinary research. It is possible because the zebrafish shares the same genes with other vertebrates, it has full operational immunologic system and embryo transparency, which can be used to monitor pathogenesis of fluorescent-labeled pathogens. Even the first obstacles connected with the small size of the zebrafish were successfully solved. Intraperitoneal and intracerebroventricular injections are possible to be carry out in adult zebrafish (Kinkel et al. 2010, Yokobori et al. 2011). Furthermore, blood sampling, which previously caused death to fish, has been also successfully mastered (Pedroso et al. 2012). Finally, the steps are taken to measure food intake in fish and improve it, as it is in rodents and other laboratory mammals (Gudmundsson et al. 2000, Volkoff et al. 2006). The small size of the zebrafish presents challenges for researchers using it as an experimental model. It is important that simple techniques are developed to enable researchers to explore the advantages of the zebrafish, which can throw new light into some processes and make them to be easier to understand. The zebrafish is also a valuable tool as a model in behavioral studies. It could become behavioral model for research of neural and genetic control of species-specific behavioral patterns connected with feeding, predator evasion, habituation and memory, or lateralized control of behavior (Miklósi et al. 2006).

Our Department uses the zebrafish as an animal model since 2005 and it was the first zebrafish laboratory in Poland. During last decade we have explored the usefulness of *Danio rerio* in the fields of neurodegenerative diseases, genetic diseases, neurobiology and behavior studies. Our zebrafish facility team members have gained their experience in leading zebrafish laboratories at various universities in Europe (Finland, Belgium, The Netherlands), where they have learnt the techniques and methods, which are used in our laboratory work. Moreover, our team members are active participants of international conferences and meetings, where they present the results of their research work and meet zebrafish scientists from all over the world. We wish *Danio rerio* became more popular among veterinary scientists, as it can complement research which, for different reasons, cannot be continued by using other laboratory animals. Especially in the field of veterinary medicine, where *Danio rerio* is also a potential host for many pathogens that are characteristic only for animals species. Currently, it has become a popular model for aquatic diseases; there is no doubt that in this case the zebrafish is a perfect organism, susceptible for various marine pathogens.

We are sure, that the zebrafish will be warmly welcomed by the veterinary researchers, as it has been previously in other areas of biomedical sciences.

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