Current knowledge on koi herpesvirus (KHV): a review

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ABSTRACT: The first outbreaks of a disease connected with high mortality of common carp and koi carp caused by koi herpesvirus (KHV) were reported in 1998 in Israel and in the United States. Since then, several cases have been confirmed all over the world. At present, this viral disease is considered to be one of the most risky factors affecting populations of common carp and koi carp. Affected fish become disoriented and swim erratically with high breathing frequency, swollen gills and partially local skin lesions. The virus was isolated from the tissues of fish showing signs of the disease and subsequently cultured on koi fin (KF-1) cells. Electron microscopic examinations revealed morphological signs identical with viruses of the family Herpesviridae. Analysis of virion polypeptides and gene DNA showed the differences between KHV and the well-known herpesvirus of cyprinids, *Herpesvirus cyprini* (CHV), and *Channel catfish virus* (CCV). Water temperature is a factor influencing the onset and severity of disease. Fish seem most susceptible at water temperatures of 18–28°C, no morbidities occur at 13°C and 30°C. At present, diagnosis of KHV is mainly based on detection of viral DNA by PCR method.

Keywords: koi; common carp; herpesvirus; koi herpesvirus

Contents

1. Introduction

A herpes-like virus designated koi herpesvirus (KHV) was first isolated in the USA in 1998 following outbreaks in koi and common carp in Israel and the USA (Hedrick et al., 2000). The first reference of a disease causing mass mortality of carp and koi carp was presented at the 9thInternational Conference of European Association of Fish Pathologists (EAFP) in 1999 (Ariav et al., 1999). It is evident the disease has a short history and therefore data on the pathogen, its occurrence, diagnosis and possible preventive measures are infrequent. Regarding possible economic losses in aquacultures, discussions on international level are now in progress about the possibility to include KHV in

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the list of contagious diseases. However, KHV had already spread extensively and due to shortcomings in diagnostic methods is not easy to determine the disease-free areas and to identify them to a high degree of certainty. On these grounds OIE and the European Community (EC) considered the possibility of notification of KHV disease and decided not to list KHV as a notifiable disease. In EU new legislation is preparing now and the next compiling of OIE and EU directions is necessary (Haenen et al., 2004). The meeting on "Assessment for OIE Listing of Koi herpesvirus disease" will hold in early May 2005.

The objective of our study is to summarize briefly significant data on this viral infection causing losses in carp cultures. The distribution is worldwide and covers a wide range of countries, from Western Europe – Belgium, United Kingdom, the Netherlands, Germany, Italy, Austria, Switzerland, Luxemburg – through South Africa to Israel, Indonesia and Japan. The occurrence of KHV infection in other Asian countries as are Philippines, Malaysia, China, Hong Kong and Taiwan is most probable. KHV outbreaks have not been yet reported in Eastern Europe where it is not a reportable or notifiable disease.

Infection caused by koi herpesvirus is highly contagious resulting in massive mortalities of koi (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio carpio*). Breeding of common carp is extensive in China and other Asian countries as well as in European countries, with yearly production reaching 1.5 million tons. Koi carp is a colour variety of common carp that was obtained by breeding. This ornamental fish, originating from Japan, is being bred in the Czech Republic since 1980s. Due to the restriction measures taken in fish trade, a less colour variety of koi carp has been imported in the recent years. Nowadays, the trade has been extended, therefore the risk of infection has increased. Although no outbreaks have been confirmed in our country so far, neither were recorded deaths of carp or koi carp with signs resembling to KHV, it does not mean that our fish cultures are free from koi herpesvirus. Rapid spread of KHV is predominantly connected with a worldwide fish trade and koi carp shows that are held without previous health examinations or requirements for health certificates. Transmission of the pathogen via live fish is a major problem of those who are responsible for guidelines concerning health controls in fish in connection with trade requirements (Hedrick, 1996). One of the preventive measures against spread of pathogens is fish inspection prior to transportation or at the production farm. Such control programmes should be based on sensitive and specific diagnostic procedures for detection of pathogens.

2. Global distribution of KHV

KHV was first diagnosed in the USA in 1998 from disease outbreaks in Israel and the USA. Current global situation is, according to the reports from the international workshop on koi herpesvirus (Haenen et al., 2004), as follows:

Country	Current situation
European countries	
Austria	the first outbreak was detected in 2003 in koi in a private pond
Belgium	the infection has been reported in koi since 1999, with mortalities up to 90%, with occasional occurrence of symptoms, outbreaks in 2002 and 2003
Denmark	since July 2002 KHV positive
England and Wales	first isolated in 2000, suspicion in 1998, 1999 and earlier in 1996, 36 outbreaks in 2002 in a wide range of carp size, the infection confirmed also in 2003
France	two suspicions in 2001, the virus was detected in koi carps imported from Israel in 2003
Germany	the first outbreak in 1997, the virus has been detected since 2002
Italy	KHV positive in 2003
Luxemburg	KHV positive in 2003
The Netherlands	first detected in 2001, positive findings since 2002
Switzerland	KHV reported outbreak in 2003, suspicion before in 2001
Poland	first detected in 2003, two outbreaks in 2004

Country	Current situation
Countries with no KHV reported	
Czech Republic	no suspicion of KHV yet
Finland	the virus has not been detected so far
Greece	no KHV has been reported
Hungary	no outbreaks so far, no suspicion
Republic of Ireland	no outbreaks so far
Russia	no suspicion, no outbreaks, but also no diagnosis
Slovenia	no KHV has been reported
Spain	no diagnosis yet and no suspicion of KHV
Sweden	no outbreaks so far
Scotland	no outbreaks so far
Asian countries	
Indonesia	KHV positive since 2002
Israel	KHV first diagnosed in 1998 after import of koi carp from Europe, outbreaks in all subsequent years
Japan	KHV positive since May 2003
Taiwan	first outbreak reported in 2002, further outbreaks in 2003 and 2004
Thailand	koi exports to Germany in 2004 were positive
Countries with KHV suspected	
China	KHV outbreak reported in 2001 in Hong Kong, handpicked koi from China were KHV positive (CEFAS) in 2002-not confirmed at source
Malaysia	koi originating from Malaysia found positive in 2001-not confirmed at source
Countries with no KHV reported	
other NACA (Network of Aquaculture Centres in Asia-Pacific) countries: Bangladesh, Cambodia, India, Iran, Korea (DPR), Laos PDR, Myanmar, Nepal, Pakistan, Republic of Korea, Singapore, Sri Lanka, Philippines, Vietnam	
African countries with KHV reported	
South Africa	outbreak reported in 2001–2003
North American countries	
USA	first outbreak 1998
South American countries	
Chile	no occurrence of KHV so far
Australian countries no occurrence of KHV so far	

3. The initial isolation of KHV and subsequent culture of the virus

KHV was first isolated in the USA in 1998 from koi experiencing mass mortalities in the USA and Israel and has been repeatedly cultured on koi fin (KF-1) cell lines since then. The new virus was not susceptible to the commonly used cell lines by fish virology laboratories. During growth KF-1 cells were incubated at 25°C, after inoculation with the virus at 20°C, cultured in minimal essential medium (MEM) with Earl's salts, supplemented with 10% foetal bovine serum (FBS), 50 IU penicillin/ml, 50 µg streptomycin/ml and 2mM L-glutamine. The virus was isolated from numerous tissues of fish with signs of the disease, including the gill, kidney, spleen, liver and intestine and induced cell fusion and intense cytoplasmic vacuolation within 5 days

after inoculation at 20°C. More complete cytopathogenic effect (CPE) was evident 7–10 days post inoculation, a complete CPE was observed 14 days post inoculation (Hedrick et al., 2000). Optimal growth of koi herpesvirus occurred at temperatures from 15 to 25°C. The highest virus concentrations were observed at 20°C at 7 days of culture. Virus detected at 4°C and 10°C and at 7 and 13 days was just above the detection limit (42$TCID_{50}$KHV/ml) of the assay. There was no evidence of virus growth at 30°C or 37°C (Gilad et al., 2003).

In Korea, koi herpesvirus from kidney and spleen of moribund fish has been cultured on FHM cell line (Oh et al., 2001). Tissue homogenate was inoculated into different fish cell lines: CHSE-214 (chinook salmon embryo), FHM (fathead minnow), EPC (*epithelioma papulosum cyprini*), and RTG-2 (rainbow trout gonad). CPE was observed only on cell line FHM, 3 to 5 days after inoculation. A complete cell lysis could be observed within 15 days. FHM cells showing CPE were examined using electron microscopy. Clear virus-like particles 70–80 nm in diameter could be detected in ultra-thin cuts of infected FHM cells.

In Germany, the pooled organ samples (kidney, liver, spleen) have been cultured on brain (CCB) tissues of carp and cell line from gill (CCG), FHM, CHSE and EPC (Neukirch et al., 1999). In CCB cells, the development of CPE became obvious 5 days after inoculation of the organ homogenate. CPE was characterized by giant syncytial formation. The syncytia spread in the cell culture during the following 4 to 5 days. In CCG cells, CPE developed since day 6 post infection. In EPC cells first syncytia could be detected from day 11 after inoculation, no cytopathogenetic effect was observed in CHSE and FHM cells.

In Israel the cocultivation of cells taken from kidneys and livers of infected fish with KF cells resulted in the appearance of CPE at 5 to 6 days post inoculation. No cythopatic effect was observed in EPC cell line (Hutoran et al., 2005).

4. KHV characterization

KHV is a dsDNA herpesvirus-like pathogen. It consists of 31 virion polypeptides and at least 8 glycosylated proteins; 12 of virion polypeptides have similar molecular weights to those of CHV-*Herpesvirus cyprini* and 10 are similar to polypeptides of channel catfish herpesvirus (CCV). The virions are composed of an inner capsid with icosahedral symmetry of approximately 100–110 nm in diameter. Mature virions contain a loosely applied envelope, giving the virion an overall diameter of 170–230 nm. The virions bear thread-like structure (tegument) on the core surface resembling those of herpesvirus. Major component of the envelope is a lipoprotein double layer. Morphology and size consistent is similar to viruses in the family of Herpesviridae. However the large size of KHV genome, which is estimated at 277 kbp, exceeds that of 250 kbp known for members of the family Herpesviridae (Ronen et al., 2003). On the other hand, genome characterization studies at the CEFAS Weymouth laboratory have identified a number of putative genes and some of these share significant homology with genes found in channel catfish virus (CCV) and other herpesviruses (Way et al., 2004). Approximately 80% nucleotide homology was established between CyHV-1, CyHV-2 and KHV. Since the viral genome sequences determined so far, including part of the thymidine kinase gene (Gray et al., 2002) (GenBank accession numbers AY208988 to -91, AJ535112, and AF411803), contain only small fragments (16 to 45 bp), similar to the case for other DNA viral genomes, it is too early

Table 1. Influence of cell lines on KHV cultivation

Cell line/20°C	Neukirch et al., 1999	Hedrick et al., 2000	Oh et al., 2001	Hutoranet al., 2005
KF-1	ND	7–10 days p.i.	ND	5–6 days p.i.
FHM	No CPE	No CPE	5 days p.i.	ND
CCB	5 days p.i.	ND	ND	ND
CCG	6 days p.i.	ND	ND	ND
EPC	11 days p.i.	transient CPE	No CPE	No CPE

ND = no data; p.i. = post inoculation

Table 2. Viral sensitivity to the temperature, pH, chemicals (Neukirch

to classify the virus phylogenetically (Pikarsky et al., 2004). At present, genome size is not currently considered a criterion for placement or exclusion of viruses in the family, the differences in sequences obtained so far for KHV have shown little similarities to those known from herpesviruses from birds or mammals. This has led to a reasonable debate on taxonomy of KHV that remains unresolved. One possibility is making large taxonomic changes in the family Herpesviridae, which would enable more precise determination of viruses such as KHV (Hedrick et al., 2004).

5. Virus impact on fish organism

5.1.Clinical signs and histopathology

Affected fish suffered from appetite loss, and may exhibit erratic swimming prior to death. The most consistent sign of the disease is discolouration, increased respiratory frequency (Gray et al., 2002), swollen, pale, patchy gills and skin lesions (Oh et al., 2001).

Histological findings reveal mass proliferations of gill epithelium with degenerative and necrotic changes, and intranuclear inclusions in the infected cells. Microscopic examinations of the liver, spleen, kidney and gastrointestinal tract show necrosis of parenchyma cells and numerous macrophages with ingested cellular debris. Neural tissue does not appear to be prominently involved in the disease process, although intranuclear inclusions in neurons may be observed in experimentally infected fish (Hedrick et al., 2000).

5.2. Virus localization in tissues

Viral DNA was detected in the kidney and blood of infected fish at very early time points after infection, as early as 3 and 5 days, respectively. Infection of fish by bathing appears to be more efficient than that by cohabitation (viral DNA was detected in the kidney and blood as early as 1 day postexposure). The amount of viral DNA in the kidney began to increase at 3 days p.i. and that in the blood began to increase at 5 or 7 days p.i. The kidney was found to be the organ in which the virus propagates most efficiently (Pikarsky et al., 2004).

PCR analysis and DNA hybridization confirmed the disseminated nature of the disease by detection of KHV DNA in the gill, gastrointestinal and liver tissues of experimentally infected fish. In contrast, viral DNA was not detected in brain tissue of experimentally infected fish by DNA hybridization and detection by PCR was not consistent, suggesting that viral DNA may be present in low copy number in the brain tissue. It is typical for some herpesviruses that do not cause pathology in neural tissue during primary infection, but rather establish latent infection in neurons. It has not been clarified yet, whether a similar situation exists for KHV (Gray et al., 2002).

5.3. The way of virus spread

The virus remains infective in water for at least 4 hours, explaining the highly contagious nature of the virus in ponds (Perelberg et al., 2003). It is not yet known if the virus enters the body of the fish through the gills or through the intestine (Hedrick et. al., 2000, Perelberg et al., 2003). It seems to be possible the virus enters the body of fish through the gills, replicates there, and induces mucosal sloughing and necrosis. The gill injury probably significantly contributes to fish morbidity. The virus replicates in the diseased gills and is then shed into the water and transferred through the white blood cells to the kidney. The virus induces there severe interstitial nephritis. This theory is in agreement with the rapid spread of this contagious disease and seems to be similar to respiratory viruses in mammals (Pikarsky et al., 2004).

Table 2. Viral sensitivity to the temperature, pH, chemicals (Neukirch, 2003)

Temperature	
inactivation	60°C
infectivity destroyed	at 35°C after 2 days
pH	infectivity destroyed at pH < 3 or > 11
Chemicals	sensitive to chloroform (and other lipid solvents and oxidising agents)

6. Temperature effects on the course of the disease

Temperature controls significantly the course of the disease in poikilothermic vertebrates (Ahne et al., 2002). Water temperature is known to influence the onset of and severity of viral infection by altering virus replication and indirectly by augmenting the efficacy of the host immune response (Alcorn et al., 2002). Water temperatures directly affect the function of both the cellular and humoral immunity. KHV outbreaks in koi and common carp are influenced by water temperature; virus concentration in the environment is not so crucial moment for infection outbreak as the temperature is. Koi carps are susceptible to very low virus concentrations (12 $TCID_{50}$KHV/ml and 1.2$TCID_{50}$KHV per ml); at 23°C mortality was 90–95%, at 18°C 90%, at 13°C no death was observed (Gilad et al., 2003). Concentrations of KHV DNA were evaluated in different tissues of virus-exposed koi held at water temperature of 13, 18, 23 and 28°C by real time PCR. Viral DNA was detected at all four water temperatures but mortality was only observed among fish at 18, 23 and 28°C (Gilad et al., 2004).

The above water temperature, used in the experiment, (13, 18, 23°C) represents seasonal variations not only in Israel, but could be applied to European countries as well. The most critical period for outbreak of KHV infections is spring when host immune response is suppressed (Fijan et al., 1971; Hedrick et al., 2000). The experimental trials demonstrated that KHV mortality experienced in spring or summer could represent activation of virus infections that were contracted earlier but were dormant at lower temperatures (13°C). Shifting of infected fish from cooler to higher water temperatures (e.g. 23°C) rapidly induced mortality (mean days to death 7–12). However, if KHV exposed fish at cooler water temperatures are held for an extended period of time (e.g. 64 days) prior to increasing water temperatures, they do not experience mortality (Gilad et al., 2003). Whether resistance was caused by acquiring immunity to re-infection is not clear as well as whether the virus in fish exposed to low temperatures for longer time is present in a latent form.

7. Diagnostic methods

Current diagnostic procedures include KHV detection based on typical clinical signs as were mentioned above. Histological examinations confirmed that massive proliferation of gill epithelia showing degeneration and necrosis and single intranuclear inclusion bodies in degenerating cells are the most prominent findings. Virus isolation in KF-1 cells is used for confirmation of etiological agent. These methods are efficient in case of fish deaths however, even in these cases virus isolation could be difficult or inefficient, if the tissue is frozen.

It seems that the current most efficient method for virus detection is PCR, which is more efficient than routinely performed isolation in KF-1 cell line. PCR detected KHV from the outbreaks in the USA and Israel showed similarity of both the isolates. The advantage of PCR is the possibility to detect viral PCR even from dead fish samples that were frozen, which failed in some cases of KF-1 cell line isolation. Amplification of viral DNA from the tissues of the gill, kidney and spleen is efficient in both field samples and in experimentally infected fish. In contrast, virus isolation using KF-1 cell line is less reliable with the gill than the kidney and spleen due to frequent bacterial contamination of gill tissue (Amita et al., 2002; Gilad et al., 2002).

Next possibility for detection and quantification of KHV DNA could be used rapid real-time TaqMan PCR assay. The new assay was specific for KHV and did not detect DNA from related herpes-like viruses found in fish (Gilad et al., 2004).

A new technique called loop-mediated isothermal amplification (LAMP) has been developed recently which can amplify and detect KHV DNA with high specificity, sensitivity, rapidity under isothermal conditions. A set of four primers, two inner and two outer, were designed based on the sequence of the thymidine kinase (*tk*) gene of KHV. This diagnostic method could be very useful in routine diagnostics and in surveillance/quarantine procedures (Gunimaladevi et al., 2004).

Another diagnostic way is detection of KHV antigen or KHV antibodies by ELISA procedures.

A diagnostic kit for rapid KHV antigen diagnosis (Enzyme Immunoassay Kit for the Quantitative Detection of KHV in Stool of Infected Fish) has been developed in Israel. It is based on the reaction of specific antibody to KHV bound in microtitre plate and a viral antigen in the intestine content of the examined fish. Evaluation is performed by spectrophotometry. The advantage of this method is a simple and rapid use; positive and negative samples are detected within few hours. Disadvantage is the fact that the kit has not been

available for routine diagnosis yet (Ko Vax, ltd., Jerusalem).

Indirect tests for KHV include ELISA testing, which looks for antibodies produced by fish against the virus (Hedrick et al., 2000). ELISA testing can provide evidence that a fish was at the same time exposed to and infected with KHV. However, as indirect tests such as ELISA cannot determine whether fish is still infected with the virus, it is not recommended as a primary diagnostic tool.

8. Differential diagnosis

Regarding to the etiological agent of the disease, which was classified into the family Herpesviridae, it is necessary to exclude cyprinid herpesvirus (CHV), the only other isolated herpesvirus from koi and common carp. Cyprinid herpesvirus was initially observed by Schubert (1966) by electron microscopy and later isolated and more thoroughly characterized by Sano et al. (1985a,b). KHV signs differ from those caused by *Herpesvirus cyprini*. CHV is responsible for both skin tumors or carp pox lesions in adult carp and dissemination form of the disease in juvenile carp. It was found that CHV cause lethal and systemic infections not only in common carp but in any cyprinid including koi carps less than two month in age (Sano et al., 1991, Calle et al., 1999).

It is possible to differentiate KHV from CHV by immunofluorescence test, when antibodies against carp herpesvirus anti-CyHV-1 do not react with KHV antigen (Hedrick et al., 2000). The differences between the two infectious agents were also confirmed by separation of proteins in polyacrylamide gel and genome sequencing by PCR (Gilad et al., 2002).

9. Seeking solutions

Besides research oriented to isolation and identification of the agent of severe affection of carp, possibilities to solve health state in cultures of carp and koi carp that would prevent waste epidemics have been sought. The greatest effort in this field has been made in Israel.

Shapira et al (2002) made experiments in crossbreeding of different carp lines and monitoring their resistance to KHV. Local carp lines (Dor-70 and Yugoslavian Nasice) and sperms of a wild sazan carp imported from the Czech Republic were used for testing. The crossbreds of local lines with sazan showed significantly lower cumulative mortality and higher survival rate after exposure to the virus compared with pure local lines or their hybrids.

Studies of Perelberg et al. (2003) were based on the fact that at each disease outbreak some fish in the stock survived and therefore a certain form of natural resistance is presumed. Their experiments with experimental infection with KHV confirmed species specificity of KHV, a possible transmission of the virus via water and existence of naturally resistant fish. Perelberg et al. stated the definition of resistant fish (RF) as those that survived at least two natural or experimental infections. The experiments were carried out with fish, survivors of the outbreak in 1999. It was found that RF will not fell ill at another viral exposure, nor at immunosuppression induced by injection of Cortisol, and that they are not carriers and no KHV DNA sequences can be detected in their tissues using PCR.

Ronen et al. (2003) found that carps may develop a natural resistance following viral exposure for 3 to 5 days at water temperature 23°C and subsequent shifting of these infected fish to higher temperature (30°C). High concentrations of specific antibodies were found in sera of these fish. An attenuated, non-pathogenic virus has been isolated, which could be used as a live vaccine to induce resistance of carp to KHV.

10. Current situation in the Czech Republic

The common carp (*Cyprinus carpio*) is viewed as majority fish for the Czech Republic. Its production in the year 2003, 2004 was 20 950 and 20 990 tons, respectively. The Czech Republic belongs to the one of the largest producers of cyprinid fish in the world. The trade with koi carps has likewise increased; that is accompanied by a higher risk of spread of this infection to the territory of the Czech Republic where koi herpesvirosis has not been recorded yet. With regard to the increasing number of European countries, where KHV has already been diagnosed, particularly the occurrence of KHV infection in the neighbouring countries – Germany, Poland and Austria, is only a matter of time before it appears in the territory of the Czech Republic. Since 2004, a grant project focused on the protection of carp breeds from KHV infection has been solved in cooperation between the Veterinary Research Institute in Brno, the University of South

Bohemia, Research Institute for Fish Culture and Hydrobiology in Vodnany and the University of Veterinary and Pharmaceutical Sciences in Brno. Within solving that project, KHV culture on KF-1 cell line and the detection of KHV DNA by PCR method has been introduced. The National Reference Laboratory for Viral Diseases in Fish is ready to examine the delivered field samples. Fish breeders have been asked to collect and send samples from such localities where health problems or signs of diseases and deaths have recently occurred. Samples from imported fish should also be sent for examination. The mentioned measures will contribute to monitoring of epidemiological situation in the territory of the Czech Republic and provide data for the introduction of national legislation, which should be in concord with the prepared legislation of the EU.

11. Conclusions

Problems concerning KHV became global at the time of the first positive findings in Japan in May 2003. At present, infection with KHV is considered to be one of the most important risk factors for koi and common carp industry with losses e.g. in Israel that exceeded 50% of the total production. Examinations of samples from different outbreaks (Germany, the Netherlands, Austria and South Africa) that were carried out in Germany revealed increasing trend in the number of positive cases which suggests a wider spread and/or greater awareness of breeders concerning KHV. Most probably cultures of both koi and common carp are endangered in Central and Eastern Europe as no measures have been taken yet, which limit carp transfer from Eastern to Central Europe (Hoffmann et al., 2004). No outbreaks of KHV have been reported in the Czech Republic and Eastern Europe currently. Spread of infection would strongly affect the new EU-member States of Central and Eastern Europe, where carp production is an economically important field of aquaculture. At national level and as a first minimal measure, KHV should be declared reportable (Schlotfeldt, 2004). Directives and guidelines are also needed for an efficient control of this pathogen in ornamental fish. First step to control spreading of the viral agent is efficient diagnosis in suppliers and buyers of fish, which would eliminate transmission of the virus into virus-free cultures.

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THE CENTAUR NETWORK

The CENTAUR network (Food and Agriculture Organization of the United Nations Established Veterinary Biotechnology and Epidemiology Network for Central and Eastern Europe) aims at upgrading the standards of economically significant priority animal diseases control in the region with particular emphasis on transboundary animal diseases, animal health and consumer protection.

The CENTAUR is willing to achieve it through dissemination of scientific information, training, links with the international centres of excellence and cooperation. The important task is also to present the problems, personalities, institutions, and scientific achievement of the region. Efficient utilization of Internet, e-mail and improvement in English language proficiency is followed, too.

Most of the Central and Eastern European Countries have been undergoing intensive socio-economic changes and is willing to join the European Union or to strengthen their trade and other relationships with the EU. Balkan countries were in the last decade the subject of a devastating war and have been intensively recovering and rebuilding a normal economy. The Network has been active since 1996 initially embracing the Czech Republic, Hungary, Poland and the Slovak Republic and since 2001 Albania, Bosnia-Herzegovina (The Federation and Republika Srpska), Bulgaria, Croatia, FYR Macedonia, Moldova, Romania, Slovenia and Turkey.

Initial links have been established with Estonia, Latvia and Lithuania who are willing to join the Network. Also in order to deal with the problems in the region more efficiently as well as for the sake of geographic integrity the CENTAUR invites the cooperation of Finland, Belorussia, Federation of Russia, Ukraine, Yugoslavia and Greece.

The CENTAUR cooperates with the FAO Network EMPRES: Emergency Prevention System for Transboundary Animal and Plant Pest and Diseases (Livestock Diseases Component), the European Commission for Control of Foot and Mouth Disease-EUFMD in Rome and with the Joint FAO/IAEA Division in Vienna. It has a link with the Regional Animal Disease Surveillance and Control Network RADISCON (NearEast/part of Africa).

Under the CENTAUR network the CENTAUR NEWSLETTER FLASH INFORMATION (CNFI), an international electronic bulletin (ISSN 1213-368X), is published, providing subscribers with instant information in the form of e-mail messages relating to fields of interest which subscribers define themselves during the process of registration. CNFI is not limited to Central and Eastern Europe but covers global animal disease-related events and is distributed to the registered readers from all over the world. The number of subscribers has been growing rapidly and new registrations are always welcome. The forms of CNFI are as follows:

E-MAIL MESSAGES are distributed to field specific registered members. Sometimes identical information is distributed to more fields of interest. Therefore second mail with identical subject and time of dispatching should not be opened but immediately deleted.

CNFI BULLETIN: approximately 10 issues per year with general information for the CENTAUR network members are distributed to all registered addresses as an attachment to e-mail. This bulletin is also available for downloading from the CENTAUR web page http://centaur.vri.cz

CENTAUR network members are welcome as authors of original papers or reviews submitted for publication in an international peer reviewed jornal for veterinary medicine and biomedical sciences Veterinarni medicina, indexed is the Web of Science, Current Contents and other databases. Papers published in this journal are free in full text at

http://vetmed.vri.cz

CENTAUR network members can request the Editor for search from the published papers if their intentions are oriented towards to contributions for CNFI or submission the manuscript for publication in the journal Veterinarni medicina.

CNFI subscription is free. Register your "fields of interest" according to the instructions available at http://centaur.vri.cz/default.asp?page=cent_reg.asp and you will receive instant confirmation of your choice by e-mail. To unsubscribe or change the selected fields of interest, send an e-mail to the CNFI editor <hruska@vri.cz>. Contributions, comments and requests of the subscribers are welcome.

CNFI and the CENTAUR network are your tools!