



## Cell culture studies on koi herpesvirus: Advancements and research trends in Asia

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**Abstract.** As viral diseases continue to threaten global aquaculture productivity, understanding host-virus interactions *in vitro* have become increasingly crucial. This review study explores advancements in koi herpesvirus (KHV) research through cell culture techniques, with a focus on contributions from Asia. KHV, a major viral pathogen affecting cyprinids, continues to challenge global aquaculture. Researchers have employed various cell lines, such as KF-1, CCB, and KCF-1, to isolate KHV strains, study, viral replication, and assess the pathogenicity of different strains. Environmental factors, including temperature and pH, significantly influence virus propagation. Diagnostic tools, including monoclonal antibodies, have been developed to detect KHV more efficiently, aiding in outbreak control. The methodology of the current study involved a bibliometric review using Google Scholar and Scopus databases to gather and analyze relevant studies. Metadata was collected, visualized, and analyzed using VOSviewer to map research trends, document origins, and key contributors. Keyword co-occurrence analysis provided insights into emerging trends in KHV-related topics. The findings underscore the importance of cell culture methodologies for developing effective KHV management strategies, improving biosecurity measures, and guiding future research on KHV control, prevention, and vaccine development. This research emphasises collaborative efforts across Asian institutions to enhance KHV research, contribute to more sustainable aquaculture practices, and improve the management of viral outbreaks.

**Key Words:** Asia, aquaculture, bibliometric, cell culture, koi herpesvirus, research trends.

**Introduction.** Global freshwater fish production has rapidly increased, with cyprinids accounting for the largest share. This growth, combined with intensive aquaculture and human-induced ecosystem stress, has led to the emergence of viral diseases and made vaccines the main tool for prevention. Previous studies focused on the epidemiology, pathology, aetiology, diagnostics, and vaccine development of koi herpesvirus disease (KHVD), as one of the main viral diseases in cyprinids (Su & Su 2018) which continues to pose challenges in aquaculture, with outbreaks as recent as 2023 (Centre for Environment and Fisheries Aquaculture Science 2023). For decades, KHVD has been classified as a serious infection by the WOA (2016) affecting common carp, koi, and ghost carp (Haenen et al 2004; Hedrick et al 2000). The causative agent, koi herpesvirus (KHV), belongs to the Alloherpesviridae family and is officially referred to as 'cyprinid herpesvirus 3' or CyHV-3 (Gotesman et al 2013; Waltzek et al 2009).

Research has demonstrated that under controlled conditions, the KHV has been isolated from infected common carp and koi using several cell lines derived from carp tissues, such as koi fin 1 (KF-1), koi fin cell (KF), common carp brain (CCB), koi caudal fin 1 (KCF-1), and koi tail 2 (KT-2). These isolations were conducted in various countries, including the US, Israel, Germany, Indonesia, Japan, Singapore, and China (Bergmann et al 2010a; Bergmann et al 2010b; Dong et al 2011; Hedrick et al 2000; Ilouze et al 2006; Neukirch & Kunz 2001; Sano et al 2004). However, only a few cell lines have successfully isolated the virus, and those lines are often challenging to manage.

Although recent reviews have been conducted, no previous bibliometric analysis has addressed the mentioned themes, making this study a groundbreaking effort in

mapping and assessing the progress of KHV-related research over the years. The results of this study are expected to highlight emerging trends and encourage further investigation into the development of using cell culture to study KHV.

**Material and Method.** A bibliometric review, which is a quantitative analytical approach used to evaluate and map scientific publications, including books, journal articles, and other scholarly outputs, to identify research trends, collaborations, and impacts within a specific field (Heersmink et al 2011), was conducted following the methodologies adapted from Fahimnia et al (2015) and Setyaningsih et al (2018).

**Research questions.** This study addresses a gap by conducting a comprehensive bibliometric analysis of the literature on the mentioned subjects, in order to answer the following questions.

1. What has been the direction of KHV research in Asia over the years?
2. How has KHV been mapped in the research topics in Asia?
3. What role have Asian researchers played in studying KHV using cell cultures (KCC)?

**Metadata collections.** Relevant documents were systematically collected in September 2024 from the Google Scholar and Scopus databases. Publications were considered relevant if they focused on koi herpesvirus (KHV) and included studies related to cell culture techniques, viral isolation, replication, pathogenesis, diagnosis, or control strategies. Only peer-reviewed journal articles and conference papers written in English were included. Google Scholar was used to gather metadata, as most KHV papers by Indonesian researchers before 2024 were published in national journals in Bahasa. Scopus was used to extend the search to include Scopus-indexed KHV-related papers, as shown in Table 1.

Table 1  
Procedures for collecting metadata in this study

| No                                                       | Databases      | Queries                                                                               | $\Sigma$ papers |
|----------------------------------------------------------|----------------|---------------------------------------------------------------------------------------|-----------------|
| 1                                                        | Google Scholar | "Koi herpesvirus" "cell line";<br>"Koi herpesvirus" "sel kultur";<br>within the title | 6<br>1          |
| 2                                                        | Scopus         | TITLE-ABS-KEY ("Koi<br>Herpesvirus") AND (cell) AND<br>Limited to Asian Countries     | 95              |
| Total number of retrieved documents from the 2 databases |                |                                                                                       | 102             |
| Total number after removing duplicates                   |                |                                                                                       | 99              |

**Data analyses.** The metadata collected in Mendeley reference manager (.ris) was visualized using VOSviewer (Van Eck & Waltman 2023) to generate network (NV), overlay (OV), and density (DV) maps. The study examined research trends, including publication characteristics, document origins, language use, global distribution of countries and institutions, subject categories, top authors, citations, and publication trends from 2004 to 2024. Keyword co-occurrence analysis was performed using the VOS algorithm as an alternative to multidimensional scaling (Putri et al 2021).

## Results and Discussion

**Publication outcomes, document types, and languages.** A total of 99 papers were identified from the initial keyword search in the Google Scholar and Scopus databases (Table 1), comprising three types of publications produced between 2004 and 2024: 97 journal articles, 1 book chapter, and 1 review, with only the journal articles included in the VOSviewer analysis. The number of publications per year fluctuated from 2004 to 2024, peaking in 2018 with 10 documents, followed by a decline and intermittent growth,

showing notable activity in 2022 (3 documents). Between the two decades from 2004 to 2024, out of 102 docs, most papers were in English (84 docs or 82%). The remaining documents were presented in Chinese (9 docs or 10%) and Bahasa (6 docs or 7%). The number of papers in Japanese and Thai was small (each of 1 doc or 0.5%).

**Distribution of publication among Asian countries.** Asian scientists from 120 affiliations contributed 99 journal articles related to the research on studying KHV using cell cultures (KCC) throughout the years (2004–2024). China produced the largest number of research outputs related to KCC over the years, with a total number of publications 44 papers (Figure 1).



Figure 1. Top five countries in Asia published KCC-related papers throughout the year.

Two institutions in Japan, the National Research Institute of Aquaculture, FRA Nakatsuhamaura, Minami-Ise, Mie, and the Japan Fisheries Research and Education Agency, led the publications on the use of cell culture to study KHV, with 14 and 13 papers, respectively. These were followed by three Chinese institutions: the Chinese Academy of Fishery Sciences (10 papers), Shanghai Ocean University (9 papers), and South China Agricultural University (8 papers). Among the 42 source titles from Asia, the Journal of Fish Diseases published the most outputs (12 papers), followed by Fish Pathology (11 papers), Veterinary Microbiology (7 papers), and both Aquaculture and Diseases in Aquatic Organisms with 5 papers each. The top five Asian researchers studying KHV using cell cultures (KCC) are listed in Table 2.

Table 2  
Top five Asian researchers studying KHV using cell cultures (KCC)

| <i>Authors</i>       | <i>Affiliations</i>                                                                              | <i>Σ papers</i> |
|----------------------|--------------------------------------------------------------------------------------------------|-----------------|
| Yuasa, Kei           | Japan Fisheries Research and Education Agency                                                    | 8               |
| Hao, Le              | GDAAS, Guangzhou, China                                                                          | 7               |
| Ke, Hao              | Guangdong Academy of Agricultural Sciences, Guangzhou, China                                     | 7               |
| Iida, Takaji         | National Research Institute of Aquaculture, FRAMinami-Ise, Japan                                 | 6               |
| Kawabata, Zen'ichiro | National Institutes for the Humanities, Research Institute for Humanity and Nature, Kyoto, Japan | 6               |





In Japan, research on KHV has examined its spread, detection, and impact on carp. In 2003, mass carp mortality in Lake Kasumigaura was linked to KHV, which was isolated from gills and kidneys (Sano et al 2004). Studies showed that KHV was susceptible to inactivation by UV light, heat, and disinfectants (Kasai et al 2005). Outbreaks were also reported in rivers with water temperatures exceeding 15°C (Hara et al 2006), while KHV was found to survive longer in filtered water, with some bacteria exhibiting anti-KHV properties (Shimizu et al 2006). Carp larvae showed resistance to KHV, whereas juveniles experienced high mortality (Ito et al 2007). Electron microscopy revealed the virus's virion formation, supporting its classification as a herpesvirus (Miwa et al 2007). KHV primarily infects the gills and spreads to other organs (Miyazaki et al 2008), while its ecology in natural waters remains under-researched (Matsui et al 2008). Diagnostic monoclonal antibodies against KHV have been developed (Aoki et al 2011), and improved methods for virus isolation, replication detection, and latency analysis have been refined (Kawato et al 2014; Yuasa et al 2012). KHV was shown to cause severe damage, leading to latency in the central nervous system (Miwa et al 2007).

In Korea, research on KHV has highlighted its severe impact on carp populations since 1998, with significant mortality peaks occurring during spring and autumn when water temperatures are between 19°C and 24°C. Although cytopathic effects (CPEs) were detected in most cases, KHV was confirmed in only 15% of the affected fish, suggesting the involvement of another pathogen (Kim et al 2010). KHV was later confirmed in ornamental koi broodstocks, where PCR detected the virus in multiple organs, showing genetic similarity to CyHV-3 (Gomez et al 2011). Further research identified key KHV proteins, such as ORF62 and ORF68, leading to the development of monoclonal antibodies for specific virus detection (Aoki et al 2011). In 1998, mass mortalities were reported across several fish farms, with *in situ* hybridization detecting KHV DNA in multiple organs (Lee et al 2012). A 2008 study identified two distinct KHV genotypes, Asia variant A1 and European variant E4, marking the first discovery of multiple KHV genotypes in South Korea (Kim & Kwon 2013) and optimal cell culture conditions for virus replication have been established (Kim et al 2020).

In Taiwan, advancements in virological studies and diagnostic tools for KHV have been notable. The KF-101 cell line, derived from koi carp fin, was cultured for over 90 passages and showed susceptibility to KHV but resistance to other viruses, demonstrating its utility in virological research (Lin et al 2013). Additionally, the monoclonal antibody MAb-B2, targeting the KHV capsid protein (ORF72), successfully detected and *neutralized* the virus in infected cells, with potential applications in diagnostic kits and vaccine development (Lin et al 2013). The ARB8 cell line, derived from *Aequidens rivulatus* brain tissue, was also susceptible to viruses like CSV and RSIV but resistant to KHV, providing further insights into fish virus studies (Yeh et al 2018). Research on the KHV ORF72 gene led to the development of the monoclonal antibody mAb 3E1, which demonstrated strong diagnostic potential by detecting KHV in fish tissues and highlighted ORF72's role in viral activity, including its mitochondrial localization and nuclear export signal (Wu et al 2020).

In Indonesia, a study in koi carp tail epidermal tissue cell cultures (KT-2) inoculated with extracts from various organs, which were incubated at 25°C for 14 days, was conducted in 2012. Cell damage was observed in cultures inoculated with extracts from gills, kidneys, and a combination of gills, kidneys, and spleen. The PCR tests confirmed that KHV caused the cytopathic effects (Sumiati & Sunarto 2012).

**Conclusions.** This study reviews advances in koi herpesvirus research, particularly in Asia, using cell culture techniques. A bibliometric analysis using Google Scholar and Scopus, visualized via VOSviewer, highlighted research trends and key contributors. Researchers employed cell lines like KF-1, CCB, KT-2, and KCF-1 to isolate KHV strains and study viral replication, with environmental factors such as temperature and pH affecting propagation. Monoclonal antibodies were developed to enhance KHV detection and control. The findings emphasize the role of cell culture in KHV management, biosecurity, and vaccine development, supporting sustainable aquaculture practices.

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