ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE

Dottorato di ricerca in Scienze Veterinarie

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Curriculum: Sanità Animale
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Spontaneous Pathology of the Aquatic Species

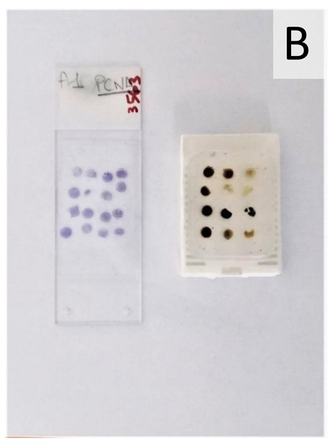
Contribution of pathology to the topic:
1) Standardization of the immunohistochemical method by tissue microarray (TMA) on koi carp (*Cyprinus carpio*) gonadic tumors. The most common antibodies tested, routinely employed by human and veterinary pathologists, cross-reacted with the neoplastic cells, as well as with the normal tissues as previously reported. TMA has been validated. Conversely, for one antibody (Placental Alkaline Phosphatase, PLAP) some tests are still running in the lab. In consideration of the results obtained during the first six months of activity, the predetermined aim was almost totally achieved.

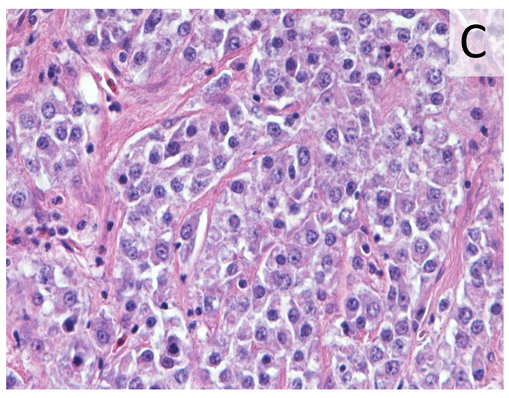
2) In adjunct to the main topic, a classic pathologic study corroborated by microbiological investigations, was carried out on koi carp (*Cyprinus carpio)*, affected by "swimbladder flooding", and on sturgeon (*Huso huso*) juveniles, which clinically shared an abnormal swimming behaviour suggesting a neuro-muscular condition. The main findings actually pointed out multiple co-infections and environmental stressors as causes of these disorders.

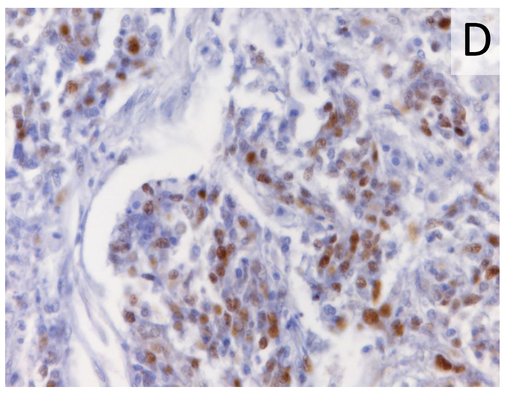
Gonadal tumors in Koi Carp (*Cyprinus carpio*)

Accepted at LXXXII SISVET/AIPVET congress

The occurrence of celomatic tumours represents a regular finding in the clinical practice, with an estimated prevalence of 1.2% in a koi population studied; among them, the gonadal tumours, and especially ovarian, are the most frequently represented (Ott Knüsel et al. 2016). Histologically, their classification is challenging, as they often present the co-existence of different cell lines, resulting in mixed tumors. Considering the difficulty to determine their exact incidence in koi carp populations, the project aims to contribute to their knowledge. During the first year of the PhD, archived cases of gonadal tumors are histologically and immunohistochemically investigated.
The aim is to describe and classify gonadal tumours by using an immunohistochemical tissue microarray (TMA) technique. Seventeen retrospective cases of koi gonadal tumors (fig. A) were evaluated according to the veterinary WHO classification and characterized with an antibody panel, vimentin, CD117, AE1/AE3 cytokeratin, E-cadherin, PCNA, by using TMA technique (fig. B).
As results 12/17 tumors were histologically diagnosed as sex cord-stromal tumors (SCST), 3/17 were mixed germ cell sex cord-stromal tumors (MGCSCST), 2/17 were germ cell tumors (GCT) (fig. C), and 1/17 was a carcinoma. AE1/AE3 cytokeratin strongly labelled the carcinoma and several SCST, but rarely GCT; E-cadherin strongly labelled almost all SCST, MGCSCST and GCT and PCNA the index of cells proliferation (fig. D). Vimentin and CD117 were only expressed in one SCST and one MGCSCST, respectively. None of the immunohistochemical markers can be considered specific for a tumor type, nonetheless, the antibodies here tested confirmed to be immunoreactive in fish tissues.



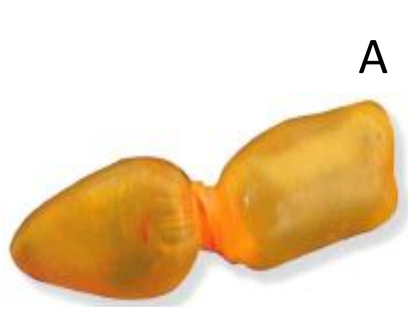


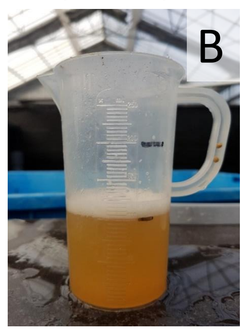


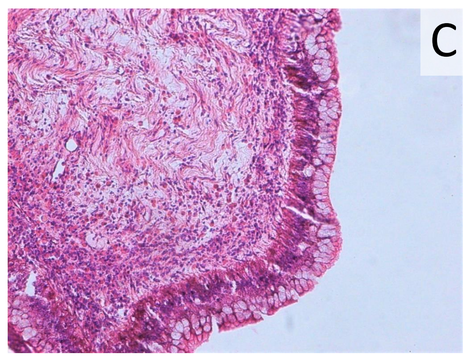
Pathological findings in Koi Carp affected with "swim bladder flooding"

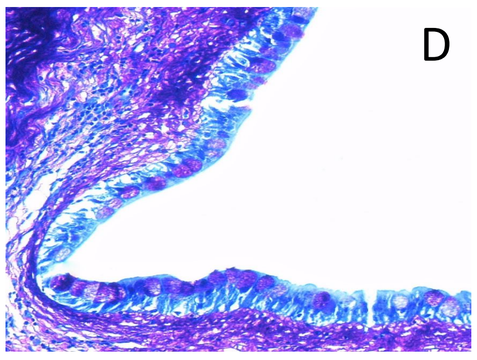
Submitted to XIX EAFP congress

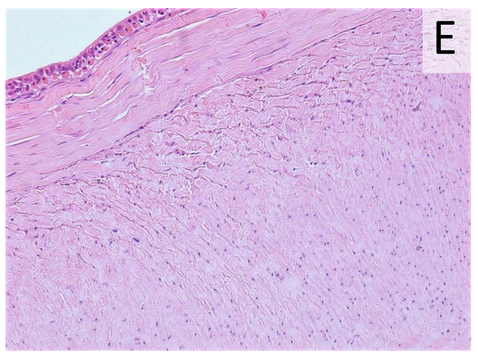
Swimbladder (SB) (fig. A) disorders include fluid accumulation, collapse, overinflation and herniation (Pees et al., 2010; Hoole et al., 2001). Possible causes of SB disorders related to infections, genetic-based anomalies, traumatic injuries and neoplasms (Holland & Frank, 2006; Holzer et al., 2014). Mycotic infections affecting SB described in other Cyprinids (Galuppi et al., 2001); literature reports in the carp are lacking. Another well-known pathology is the Swim Bladder Inflammation (SBI), grossly associated with reddish-brown exudate and etiologically ascribed to *Sphaerospora dykovae* (Chang et al, 2016). The aim of this study is to deepen the knowledge of SB disease in koi affected by abnormal swimming behaviour and loss of the neutral buoyancy.
The aim is to investigate koi carp with clinical signs suggesting swim bladder (SB) disorders.
Eight koi carps, clinically examined for abnormal swimming behaviour were studied. SB (fig. B) fluid was detected and sampled with ultrasound-guided fine needle aspiration. Four cases were cytologically and bacteriologically investigated. The SBs of 6 fishes were submitted for histology.
Subgrossly, the thickness of the SB walls ranged from 0.7 to 7.7 mm. Histologically 5 SBs showed chronic aerocystitis. In 3 cases, granulation tissue with a moderate number of macrophages (fig. C) associated with mucous or squamous metaplasia (fig. D) of the epithelium was present; in 2 cases fibrosis (fig. E) of the wall was detected. One case showed mycotic aerocystitis (fig. F).
Histological investigations showed a chronic inflammation and adaptive responses.

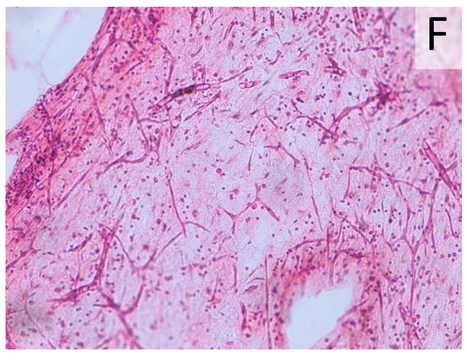












Multiple co-infections and environmental stressors as causes of chronic mortalities in juvenile sturgeons (*Huso huso*)

Submitted to XIX EAFP congress

The Acipenserid belonging to the species *Huso huso* (beluga) reared for different purposes, depending on the commercial supply, for meat and caviar production or for replenishing the wild stocks.
Literature reports of *Huso huso* or their hybrids outbreaks are limited to bacterial infections (*Vibrio vulnificus; Aeromonas hydrophila* and *Yersinia ruckeri*) (Khalil et al., 2015; Santi et al., 2018).
In fish farms, a significant percentage of Siberian sturgeons *Acipenser baerii* mainly from 3 years old onwards, exhibited anomalies of their vertebral skeleton. This pathology lead to death before being mature enough to produce caviar, resulting in important financial loss for farmers (Sire and Leprevost, 2018). Spinal anomalies in the wild or in rearing conditions of Acipenseriformes and the determinism of these pathologies is largely unknown (Leprevost and Sire, 2014). Athanassoupoulou et al (2004) reported that juvenile sturgeons reared in open-flow systems were susceptible to skeletal abnormalities of the spine; histopathological studies were inconclusive for what concerned the etiology, but anomalies seemed related to horizontal transmission of nodavirus by infected sea bass.
The aim is to contribute, with the gross and histopathological findings, to the description of an episode of chronic mortality of *Huso huso* juveniles that were submitted to the service of Anatomic Pathology of DIMEVET. Cyto-spin was performed from celomatic effusion. Main organs were sampled for histology. Animals showed neurologic signs such as abnormal swimming (inverted or circular), sudden movements, hyperactivity to stimuli alternated to prolonged resting on the bottom or laying on side. Two cases presented U-shaped body (fig. A), one case showed epaxial muscles softening, and three cases had multifocal ulcerative dermatitis. Three cases had a sero-hemorrhagic celomatic effusion which was septic in one case. Histology showed, degeneration and atrophy of epiaxial muscles (fig. B) in animals presenting U-shaped body and muscles softening. One animal showed a systemic mycosis (fig. C, D). The histological findings were variable among the animals studied; it is suggested that predisposing environmental stressors may have facilitated the clinicopathological onset.