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Occurrence and characterization of *Salmonella* strains isolated from animals involved in animal assisted interventions (AAIs)

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Purpose: The Animal Assisted Interventions (AAIs) are constantly increasing worldwide and require very close animal-human contacts. The surveillance of zoonotic microorganisms in these animals should be compulsory, especially when patients are immunocompromised.

The aims of this study were to investigate the potential role of animals involved in several AAIs as carriers of *Salmonella* spp. and the phenotypic and genotypic characterization of isolates.

Methods & Materials: A total of 250 faecal samples from 13 animal species involved in AAIs (94 equids, 56 dogs, 40 birds, 37 rabbits, 20 cats and 3 other species), were collected. *Salmonella* spp. was detected by ISO 6579:2002 protocol. The isolates were serotyped by the OIE Reference Laboratory for salmonellosis (IZSVe, Italy). The phenotypic resistance profile of isolates was assessed by broth microdilution method to define the minimum inhibitory concentrations (MICs) (according to the CLSI guidelines), whereas molecular typing was determined by Multilocus Sequence Typing (MLST) and microarray analyses (Alere Technologies, GmBH).

Results: Of the 250 animals tested, 6 (2.4%) cats (of a single Association) were positive for *Salmonella* spp. Serotyping showed the presence of *Salmonella* Rissen and *S.* Typhimurium monophasic variant 4,[5],12:i:-. All the isolates showed phenotypic resistance to Î²-lactams, phenicols, aminoglycosides, tetracyclines, folate pathway inhibitors and fluoroquinolones. Most of the isolates carried genetic determinants conferring resistance to Î²-lactams, aminoglycosides, sulphonamides and trimethoprim.

Conclusion: The animals involved in programs of AAIs need to be well cared of and fed with safe diet. The 6 positive cats were usually fed with dry and canned commercial food but also, occasionally, with raw meat-based products (Bone And Raw Food - BARF), that could be the source of the observed infections. The Italian national guidelines on AAIs suggest avoiding the use of raw food to feed the animals involved in AAIs. The finding of the zoonotic multidrug-resistant bacteria suggests that the animal handlers and the volunteers should be adequately trained and that systematic surveillance should be performed as part of the screening process for animals involved in AAIs.

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20.123

The clinical epidemiology of acute flaccid paralysis in Australian children from 2007 to 2017

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Purpose: The Paediatric Active Enhanced Disease Surveillance (PAEDS) network conducts Australian acute flaccid paralysis (AFP) surveillance as part of the global effort to eradicate Poliovirus. We aimed to describe the clinical epidemiology of childhood AFP over 10 years captured by PAEDS. We hypothesised there would be no secular or seasonal trends evident.

Methods & Materials: Data were collected by PAEDS nurses at five Tertiary Paediatric hospitals from 2007 to 2017. Children aged 0-15 years with AFP-like symptoms were included. For analysis, two PAEDS datasets were unified, with AFP compatible cases stratified by demographic features and expert review panel diagnoses.

Results: Of 400 AFP compatible cases, 50.7% were male; 45% of cases were children aged 0-4 years, 7.3% aged <1 year. As expected, the most populous states Victoria (n = 147) and NSW (n = 128) reported the majority of cases. The most frequent diagnoses were Guillain-Barre Syndrome (GBS; 33.8%), Acute Disseminated Encephalomyelitis (ADEM; 18.3%) and Transverse Myelitis (TM; 17.3%). A variety of less common diagnoses also caused AFP; some showing age-related predominance. No secular trend was seen across all AFP cases; however there was an apparent increase in both TM and ADEM. ADEM was more frequent in winter and TM in autumn. For the three major diagnoses, 14% of cases documented a concomitant infectious organism.

Conclusion: There is a stable annual incidence of AFP in Australia children between 2007-2017 despite increasing rates of TM and ADEM. AFP diagnosis is age-related. Our ongoing work will describe the clinical features of the major diagnostic groups, in particular analysing for disease clusters that may represent previously unrecognised outbreaks of neurotropic infection (e.g. non-polio enteroviruses).

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20.125

Occurrence of *Clostridium difficile* in dogs involved in animal assisted interventions (AAIs) In Italy

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Purpose: *Clostridium difficile* is the most important infectious agent of healthcare-associated diarrhea in patients. In the last years, there has been an increasing of community infections due to strains characterized by high virulence and/or antimicrobial resistance. Even if transmission of *C. difficile* from animals to human has not yet been demonstrated, recent studies suggest animals as possible reservoirs for human pathogenic strains.

AAIs are a multidisciplinary support, beneficial to humans and based on a closed human-animal interaction. AAIs are developing considerably in Italian healthcare facilities involving especially dogs. Given the frequent involvement of vulnerable population groups, there is a need of a deeper knowledge about the role of animals involved in AAIs as possible carriers of zoonotic or antimicrobial resistant agents. This is mandatory for an effective risk assessment to protect both the humans and the animals involved

The aim of this study was to evaluate the presence of *C. difficile* in healthy dogs involved in AAIs.

Methods & Materials: 56 faecal samples from healthy dogs involved in AAIs in Italian healthcare facilities were collected. Isolation and identification of *C. difficile* were performed according

to standard methods and the isolates were confirmed by PCR and characterized by agarose gel-based PCR-ribotyping method previously described.

Results: *C. difficile* was isolated in two out of 56 (3.6%) of the healthy dogs tested. Those dogs were involved in AAIs with children, disabled and elderly people. Two different PCR-ribotypes (RT) were identified: RT078 and RT087.

Conclusion: The PCR-ribotypes of *C. difficile* detected in previous studies in dogs were RT001, RT009, RT010 and RT014. RT078 and RT087 were commonly isolated from humans and sometimes from other animal species than dog; especially RT078 is considered an emerging cause of human infections in the USA and in Europe, being the 3rd most common type detected in hospitals. RT087 was identified as the predominant RT in a Hungarian hospital epidemiological study.

Our results suggest that dogs may be a source of these ribotypes for humans and raise the question of the zoonotic potential of *C. difficile*. The role of dog-human interaction in the cross-transmission of this agent should therefore be further investigated.

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20.127

Host's immunogenetic risk factors in patients with Chagas Disease

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Purpose: Chagas Disease (CD) is endemic in many Brazilian regions being considered an important public health problem. About 30% of chronically infected individuals develop Chronic Chagas Cardiomyopathy (CCC) and 10% develop Disgestive Tract Disease (DTD) after 20 of infection by the Protozoan *Trypanosoma cruzi*. Taking into account that the host immune response might be modulated by different genes, it is expected that immunogenetic markers may at least partially explain this phenomenon. This study investigated the levels of CCL3 and CCL4 chemokynes, the IL17 cytokine genotypes, CCR5Î"32 and CCR5 59029 A/G Polymorphism, the functional MICA-129 Polymorphism in patients with different clinical forms of CD.

Methods & Materials: Patients chronically diagnosed with CD and controls were recruited over a period of eight years. CC and DTD were diagnosed by clinical and laboratory routine procedures and the chronic infection (IgM, IgG anti-*T. cruzi*) was assessed by ELISA. PCR-RFLP was used to identify MICA, CCR5 polymorphisms and IL-17 alleles. CCL3 and CCL4 chemokines were measured using Milliplex MAP Assay. The data were analyzed chi-square. Odds Ratio and confidence interval at 95% values were calculated ($p < 0.05$).

Results: CCL3 and CCL4 plasma levels and the CCR5 59029 A/G polymorphism were not correlated with different clinical forms of CD. On the other hand, the A allele and A/A genotype of IL17A as well as the functional MICA-129 Polymorphism were significantly increased in patients with severe Left Ventricular Systolic Dysfunction (LVSD) when compared to controls.

Conclusion: In conclusion the severity of LVSD is strongly influenced by genes involved in different steps of innate and adaptive immune response against *T. cruzi*.

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20.128

Fetal death caused by *Toxoplasma gondii* infection

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Purpose: Molecular biology is a really good tool worldwide being used supporting the diagnosis in infectious disease, specially among that potentially congenital transmited. Toxoplasmosis is a worldwide spread infection, causing with clinicals aspects reported being different among populations. Here we describe clinical aspects presented by newborn babies with congenital toxoplasmosis from SÃ£o Paulo state, Brazil.

Methods & Materials: From the 45 pregnant women with previous *Toxoplasma gondii* acute infection, were collected amniotic fluid to determine fetal infection by *T. gondii* by PCR, using *B1* gene as molecular marker and blood to new serology by ELISA. At birth were noted the gestational age and the babies weight. Following WHO protocols prematurity was defined as gestational age <37 weeks and low birth weight â‰¤2499 grams. The *t*-test was used to compare values ($p < 0.05$).

Results: The fetal infection determined by positive PCR in amniotic fluid was found in 28 (62.2%) samples. The *T. gondii* antibodies were reassayed; from that still 33 IgM positive samples 19 (57.6%) were positive PCR; from the 12 IgM negative 8 (66.7%) were positive PCR. From the total 11.1% (5/45) newborns present symptons related to toxoplasmosis at birth and the fetal infections were determined in 3 of them. Fetal deaths accounted for 11.1% (5/45) and 8.8% (4/45) of the positive PCR and 2.22% (1/45) negative PCR. The maternal age, prematurity and low birth weight were not related to fetal infection ($p = 0,675$; $p = 0,941$; $p = 0,697$, respectively.

Conclusion: Congenital toxoplasmosis was the cause of fetal death. Prematurity and low birth weight are not related to congenital infection by *T. gondii* in Brazil as has been reported in other countries. The results confirmed that there was vertical transmission of *T. gondii* determined by PCR.

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