ImmunoElectronMicroscopy (IEM) detection of viral agents in diarrheic pigs during the period 2002-2008 in Northern Italy

A. Lavazza, C. Tittarelli, M. Cerioli, Alborali G. L., P. Cordioli

Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna "B.Ubertini"
via Bianchi 7/9, 25124, Brescia, Italy

antonio.lavazza@bs.izs.it
Keywords: pig, enteritis, virus, diagnosis, Italy

Enteric diseases are important economic problems affecting pig production. Multiple enteric infections can occur, often in association with non-infectious factors, giving rise to complex disease patterns, with difficulties to establish successful control measures. Viruses can play an important role by modifying the intestinal mucosa and thus predisposing to secondary infections. The virologic diagnosis may be difficult since many enteric viruses of pigs (i.e. torovirus, sapovirus, rotavirus non-A etc.) can not be isolated *in vitro*, thus the most used methods are negative staining electron microscopy (nsEM), ELISA and PCR. Among EM methods, immuno-electronmicroscopy (IEM) is the more sensitive and specific. Indeed, using respectively convalescent or hyperymmune sera, unknown or not suspected viruses and more viruses in association can be detected. The aim of this study is to report the data of identification by IEM of viral agents in diarrheic pigs during the period 2002-2008 in Northern Italy.

A total of 3878 examinations were performed on faecal or intestinal samples from pigs with enteric disease. For IEM examination, intestinal contents or faecal samples were suspended in distilled water and then treated using the Airfuge ultracentrifugation method [2]. The list of sera used in IEM included both hyperimmune sera produced against reference strains: BoTV (Bredavirus), PCV type 1 and 2, PEDV, TGEV, Group A Rotavirus, Pig Enterovirus as well as convalescent sera against known (PCV + PEDV) or unknown agents (convalescent sera from recovered animals after a field outbreak). Not all the samples were tested using each serum but the different sera were employed according to the clinical suspect and anamnestic data available. Each serum was previously serially diluted to ascertain its optimal titre giving a clear immunoaggregation of viral particles. The grids stained with 2% NaPt were observed with a TEM Philips CM10 at 19-25000x. The identification of viral particles was achieved on the basis of the typical morphological characteristics of each viral family. Both immuno-aggregated particles as well as other virions not clumped by the used sera were detected.

During the period 2002-2008, 1449 (37.4%) of 3878 examinations were positive for one or more viruses; the negatives were 2429 (62.6%) The results are shown in table 1 and even when present as viral association, the count and percentage of each virus are referred to the total of positive exams. Rotavirus (12.5%) and Coronavirus of PED (17.3%) represented the most common viral agents. A total of 553 virions (14.3%), mostly small round faecal viruses, were not fully classified due to their not well defined morphology. In Figure 1 are reported some examples of virions observed at EM in samples of diarrheic pigs.

This study shows that nsEM and particularly IEM could be used for the diagnosis of enteric viruses in diarrhoeic pigs since they permit to detect not cultivable virions and multiple viral infections. In addition EM could give a good diagnostic indication in a very short time (few hours) and it gives valuable indication for further investigation. About the prevalence of different viruses it is confirmed the importance of rotaviruses as primary agents

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-367, © Verlag der TU Graz 2009

of enteritis and their steady incidence. The comparison with type A ELISA test (data not shown) indicated that many strains belongs to non-A types and the studies performed on these isolates confirmed a high genomic variability [3]. PEDV was constantly found and is endemic but it periodically increases during cold months on 2002-2003 and on 2005-2006 when several outbreaks were reported [4]. PCV was detected in the gut contents and such evidence has been related to the pathogenesis of the infection [1]. Many not classified agents (i.e. enterovirus-like and calicivirus-like) need to be further characterized to better define their correct classification. Similarly the pathological significance of some agents (i.e. torovirus, adenovirus, parvovirus) has to be fully ascertained.

1. Cerioli M. et al., Proc. 4th International Symposium on Emerging and Re-emerging Pig Diseases. Rome, 29 June-2 July 2003, p224-225.
2. Lavazza A. et al., Proc. 14th International Pig Veterinary Society. Bologna, Italy, 7-10 July 1996, p. 131.
3. Martella V. et al., Virology 346 (2006) p301-311.
4. Martelli P. et al., Veterinary Record 162(10) (2008) p307-310.

Year		2002	2003	2004	2005	2006	2007	2008	Total
Total exams		716	788	622	384	746	333	289	3878
Neg	N°(%)	407(56.8)	399(50.6)	412(66.2)	288(75.0)	489(65.5)	220 (66.1)	214 (74.0)	2429(62.6)
Adenov.	N°	3(0.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	4(0.1)
Caliciv.	N°	3(0.4)	2(0.3)	6(1.0)	0(0.0)	5(0.7)	3(0.9)	0(0.0)	19(0.5)
Coronav.	N°	152(21.2)	224(28.4)	71(11.4)	38(9.9)	152(20.4)	33(9.9)	1(0.3)	671(17.3)
Circov.	N°	54(7.5)	107(13.6)	0(0.0)	0(0.0)	2(0.3)	3(0.9)	0(0.0)	16(0.4)
Enterov.	N°	16(2.2)	2(0.3)	0(0.0)	0(0.0)	15(2.0)	19(5.7)	0(0.0)	52(1.3)
Parvov.	N°	2(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(0.1)
Rotav.	N°	59(8.2)	99(12.6)	123(19.8)	40(10.4)	56(7.5)	55(16.5)	53(18.3)	48 (12.5)
Torov.	N°	4(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(0.1)
Nc°	N°	155(21.6)	245(31.1)	43(6.9)	31(8.1)	32(4.3)	12(3.6)	35(12.1)	553(14.3)

Table 1. Results of EM (°Nc= not classified, including small round faecal virus)

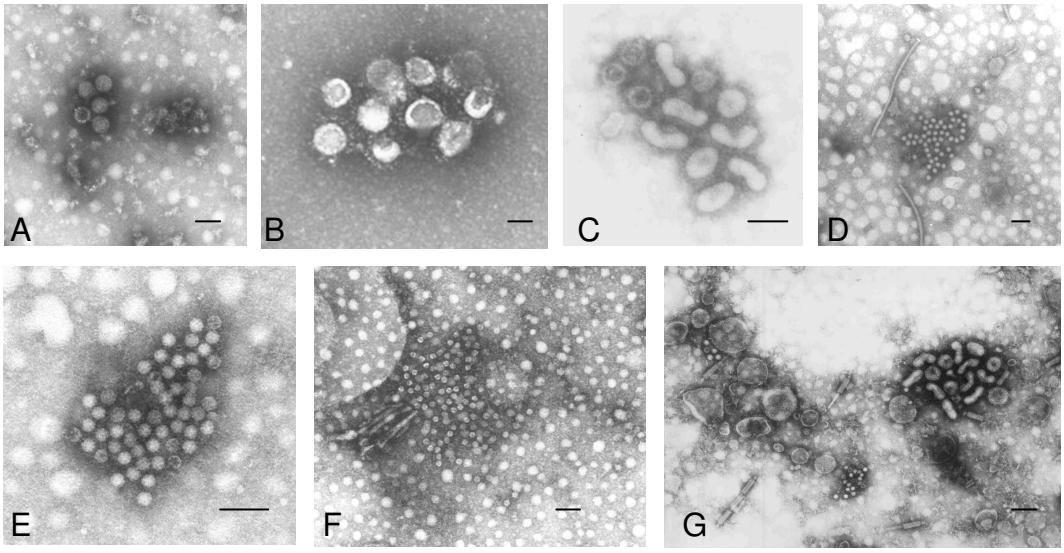

Figure 1. EM pictures of virions from enteric pigs (nsIEM-NaPT 2%) Bar = 100nm A=rotavirus; B=PEDV coronavirus; C=rotavirus associated to torovirus; D=enterovirus-like; E=calicivirus; F= circovirus; G = torovirus associated to enterovirus-like and circovirus.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-367, © Verlag der TU Graz 2009