Journal of Infection 79 (2019) 462–470

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letters to the Editor

Diagnostic accuracy of 16S rDNA PCR in bacterial pyomyositis: A prospective study

Dear Editor,

In this Journal, Lelouvier and colleagues reported the use of 16S metagenomic sequencing to diagnose polymicrobial and culture-negative bacteraemia. [1] We also used 16S rDNA PCR to study the bacteriology of bacterial pyomyositis, a rare infection of skeletal muscles in which cultures are often sterile as most patients are receiving antibiotic therapy for days or even weeks prior to aspiration of pus.[2] Indeed, establishing the diagnosis is a clinical challenge as initial symptoms are nonspecific and last for a mean duration of 23 days before hospitalization.[2] Aspiration of purulent material – the diagnostic gold-standard – is required for culture-based identification of the causative agent.[3] Staphylococcus aureus is the most common pathogen reported in published cases, but other bacteria are frequently identified and empirical treatment is typically with broad-spectrum antibiotics.[4] Empirical treatment is tenuous, however, because the authentic pyomyositis epidemiology is not known. All reported cases are individual clinical cases and no prospective study has been carried out allowing the evaluation of the frequency of this disease in the community at large or of the proportion of culture-negative cases.

Broad-range PCR targeting 16S ribosomal DNA (16S rDNA PCR) allows the identification of bacterial pathogens in culture-negative clinical samples[5] and is particularly useful in patients who have been treated with antibiotics.[6,7] In the present prospective study, we compared results of 16S rDNA PCR and culture in patients with pyomyositis. We prospectively included all patients admitted to our Infectious Diseases Unit suspected to have pyomyositis who underwent needle aspiration of pus from muscle for microbiological diagnosis. The purulent material was distributed among three tubes, for microscopic analysis, routine culture (at CHU de Bicêtre) and 16S rDNA PCR analysis using the SepsiTest kit™ (Molzym) according to the manufacturer's instructions (at HEGP). PCR analysis was performed blinded to the culture results.

Between 2008 and 2018, 12 patients were recruited (7 female, 5 male); their mean age was 58 years (range: 34–89). Seven (58%) patients had an underlying condition or disease (Table 1): haemodialysis ($N=2$), HIV infection ($N=2$), cancer ($N=2$), cirrhosis ($N=1$). For the five remaining patients, four were without notable medical history and one had *Streptococcus agalactiae* endocarditis 6 months before admission. CT-scans revealed pus in the psoas ($N=5$), lower limb muscles ($N=5$), superior limb muscles ($N=1$) or lumbar muscles ($N=1$). At the time of pus aspiration, all patients were receiving antibiotic treatment with a median duration of 23 (range: 3–94) days. Symptoms of muscle infection manifested in only 5 (42%) cases, lasting for a mean of 23 days before the definite diagnosis was made.

16S rDNA PCR performed on the pus allowed bacterial identification in all cases: *Staphylococcus aureus* ($N=4$); *Streptococcus* species ($N=3$), *Clostridium* species ($N=2$), *Arcanobacterium haemolyticum* ($N=1$), *Proteus mirabilis* ($N=1$) and *Enterococcus* faecium ($N=1$).

Cultures of pus samples from 5 (42%) patients grew bacteria that were identified in agreement with those identified by 16S rDNA PCR (Fig. 1), i.e., *S. aureus, P. mirabilis, C. septicum, A. haemolyticum* and *E. faecium*. For four (33%) patients, pus cultures were sterile (Fig. 1), but concomitant cultures of blood or synovial fluid samples yielded bacteria concordant with those identified by 16S rDNA PCR, i.e., *S. aureus* ($N=3$) and *S. pyogenes* ($N=1$). We considered these bacteria to be the causative agents of pyomyositis and treated the patients accordingly, with favorable outcome in each case.

For the 3 (25%) remaining patients (Fig. 1), microbiological analysis, but not 16S rDNA PCR, yielded negative results. Their medical history agreed with the 16S rDNA PCR findings. Patient 10 had been treated for *S. agalactiae* endocarditis 6 months prior to the onset of pyomyositis. Patient 11 fully recovered after 8 weeks of amoxicillin and clindamycin therapy, suggesting that the *Streptococcus* identified by 16S rDNA PCR was the cause of pyomyositis. Patient 12 was admitted for fever of unknown origin. A CT-scan showed a gas-containing iliac abscess. Cultures of purulent aspirate were negative, but 16S rDNA PCR revealed the presence of *C. perfringens*. Piperacillin-tazobactam was administrated. Cultures of a second percutaneous aspirate were also negative, while the 16S rDNA PCR again confirmed the presence of *C. perfringens*. Unfortunately, the patient died from unrelated hemorrhagic shock.

As pyomyositis is a rare infection, we could only recruit 12 patients for the present study. This study is, however, one of an unbiased, continuous series that allowed us to evaluate 16S rDNA PCR in both culture-positive and -negative pyomyositis. The high prevalence (70–90%) of staphylococci in bacterial cultures of aspirates from muscle could result from greater fitness of staphylococci to survive in muscle abscesses despite prior antibiotic therapy.[2,3] Rampini et al. have shown the interest of 16S rDNA PCR in the identification of bacterial pathogens responsible for infections in patients undergoing antibiotic therapy, particularly when samples are retrieved from a usually sterile compartment.[6] The use of 16S rDNA PCR on valves of patients with culture-negative infective endocarditis is now well recognized.[5]

In conclusion, the results of 16S rDNA PCR were in agreement with those of routine culture in all culture-positive cases. When cultures were negative, species identification by PCR guided a switch in treatment, from broad- to narrow-spectrum antibiotics. This is of major interest as most pyomyositis cases occurs in patients with chronic underlying disease predisposing to healthcare-associated infections, and choosing the appropriate antibiotic therapy is a particular concern in the era of multiresistant

https://doi.org/10.1016/j.jinf.2019.08.008
0163-4453/© 2019 The British Infection Association. Published by Elsevier Ltd. All rights reserved.

Table 1
Patient data, antibiotic treatment, bacterial identification and outcome.

N	Sex/age (y)	Medical condition/clinical presentation before diagnosis of pyomyositis	Localization of pyomyositis	Initial antibiotic regimen	Duration of antibiotic therapy before muscle pus aspiration (d)	Culture results of muscle pus	Culture results of other samples	16S rDNA PCR result from muscle pus	Adapted antibiotic therapy and outcome
1	F/52	• No underlying disease • Fever and pain in the left thigh for 10 days	Left quadricipital muscle	AMX/Clav (2 d), OXA (10 d), LVX (3 d)	15	*Staphylococcus aureus*	None	*S. aureus*	LVX (16w) Recovered
2	F/63	• Hemodialysis • Fever and pain in the left leg for 10 days	Left calf	LZD (2 d), AMX + LZD (2 d), AMX (10 d)	14	*Proteus mirabilis*	None	*P. mirabilis*	OFX (12w) Recovered
3	M/87	• Prostate cancer in remission for 10 years • Fever for 16 days	Left quadricipital and psoas muscles	AMX/Clav (10 d)	10	*Arcanobacterium haemolyticum*	None	*A. haemolyticum*	LVX prolonged antibiotic therapy Recovered
4	M/45	Active bronchial cancer with metastasis	Left psoas	Vancomycine (17 d)	17	*Enterococcus. faecium*	*E. faecium* in blood culture	*E. faecium*	Targocid (6w) Recovered
5	M/57	• HIV, active colon cancer • Fever and mild pain in the right arm for 15 days	Right tricipital muscle	CRO + MTZ (9 d), TZP (7 d), TGC (7 d), AMX (16 d)	39	*Clostridium septicum*	*C. septicum* in blood culture	*C. septicum*	AMX/Clav (8 d) Recovered
6	F/41	• No underlying disease • Rhinoplasty with post-operative fever for 28 days	Both thighs and legs	LZD + AMX (10 d), AMX/Clav + CLI + GEN (10 d)	20	Sterile	*Streptococcus pyogenes* in blood culture	*S. pyogenes*	AMX + CLI (3w) Recovered
7	M/34	• Vertebral fracture T12 Arthrodesis T10-L1 in 2001.	Lombar	CRO (1 d), TZP (2 d), CZ (10 d)	13	Sterile	*Staphylococcus aureus* in blood culture	*S. aureus*	OFX + R (4w) Recovered
8	F/ 62	• Alcoholic cirrhosis, insulin-treated diabetes • Fever and chills	Left psoas and iliac muscles	OXA + GEN (4d), OXA (8 d)	12	Sterile	*Staphylococcus aureus* in blood culture	*S. aureus*	LVX + R (42 d) Recovered
9	F/38	• HIV infection • Arthritis of the left knee and pain in the left calf with fever for 30 days	Left quadricipital muscle	OXA (28 d)	28	Sterile	Negative blood culture, but culture of synovial fluid grew *S. aureus*	*S. aureus*	OXA + LVX (4w) Recovered
10	F/89	• Bilateral hip prosthesis • *Streptococcus agalactiae* endocarditis of the mitral valve 6 months before admission • Isolated fever for 28 days	Bilateral psoas and gluteus maximus, communicating with hip prothesis	AMX (6w), CRO (6w), CRO + MTZ (10 d)	94	Sterile	Negative concomitant blood culture, but *S. agalactiae* isolated 6 months earlier	*S. agalactiae*	AMX prolonged antibiotic therapy Recovered
11	F/58	• No underlying disease • Fever and pain in the left thigh for 11 days	Left quadricipital, obturating and gluteus muscles	AMX/Clav (3 d)	3	Sterile	None	*Streptococcus spp.*	AMX + CLI (8w) Recovered
12	M/71	• Hemodialysis • Fever for 10 days	Left ilio-psoas muscle	HRZ + CLARI (8 d), MTZ (3 d)	11	Sterile	None	*C. perfingens* in both pus	TZP (19 d) Deceased

Abbreviation: AMX, amoxicillin; AMX/Clav, amoxicillin/clavulanate; CLARI, clarithromycine; CLI, clindamycin ; CRO, ceftriaxone; CZ, cefazolin ; FEP, cefepim; GEN, gentamicin; H, isoniazid; IPM, imipenem; LVX, levofloxacin; LZD, linezolid; MTZ, metronidazole; OFX, ofloxacin; OXA, oxacillin; R, rifampicin; TGC, tigecycline; TZP, piperacillin-tazobactam; Z, pyrazinamide; . (d), days. (w), weeks; (y), years.

bacteria.[3] We suggest that16S rDNA PCR be applied in all cases of pyomyositis, particularly when bacterial cultures are negative. In the future, an authentic microbial epidemiology of pyomyositis could be described if bacteria recovered from culture-negative pus samples were included.

Declaration of Competing Interest

The authors have no conflicting interests with respect to this manuscript.

Acknowledgments

The authors are indebted to the colleagues who cared for our patients and to those who have referred their patients to our unit for the management of infection.

This work has been presented at the 28th European Congress of Clinical Microbiology and Infectious Diseases, Madrid 2018

We received no financial support for this study.

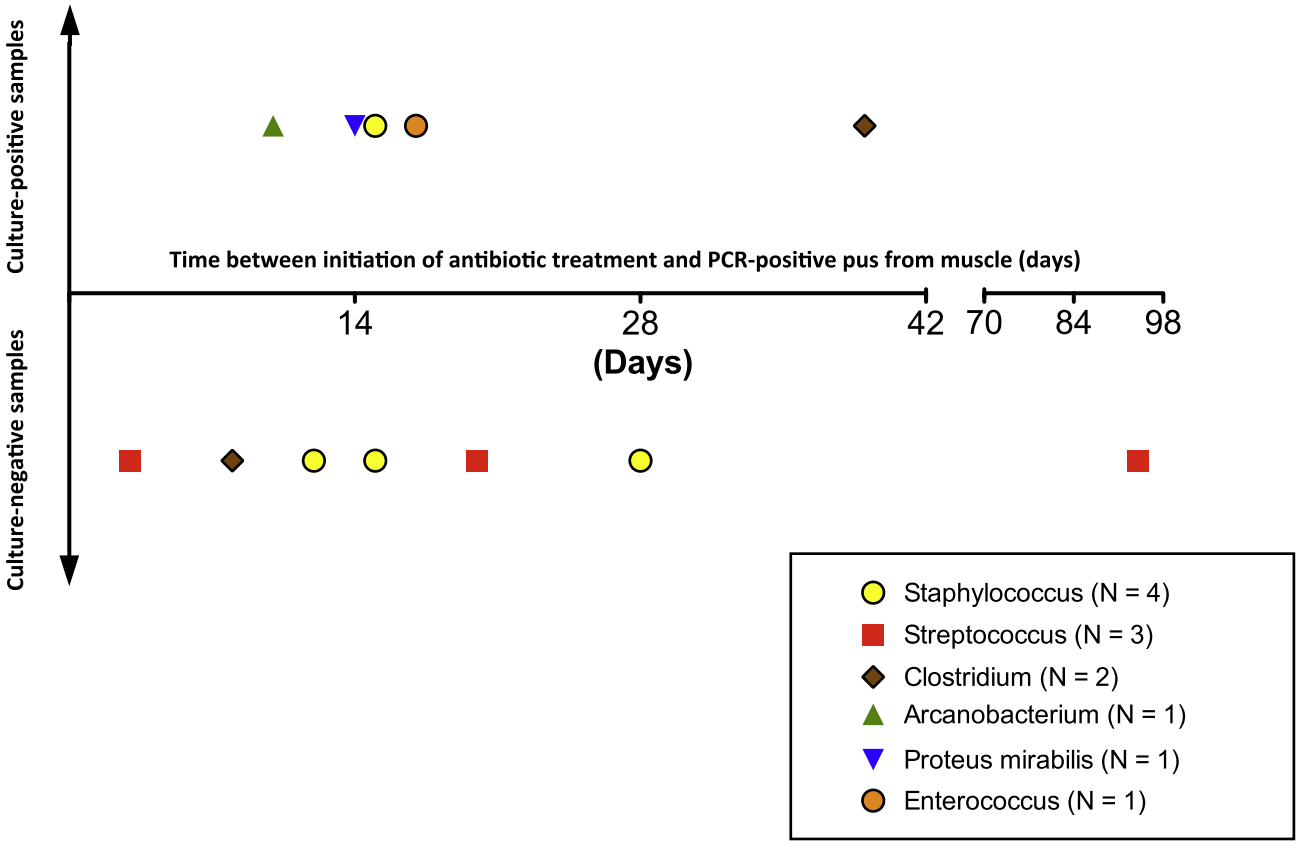

Fig. 1. Results of culture (positive or negative) plotted against 1 the time between initiation of 2 antibiotic treatment and PCR-positive pus from muscle (days). This figure shows that a routine 3 bacterial culture grew in only 5 (42%) cases (Top Figure), while 16S rDNA PCR identified 4 bacteria in 7 additional cases (58%) (Bottom Figure). It is noteworthy that streptococcus species, 5 known to be difficult to culture if the patient is under antibiotic therapy, were only identified by 6 16S rDNA PCR.

References

1. Lelouvier B., Servant F., Delobel P., Courtney M., Elbaz M., Amar J.. Identification by highly sensitive 16S metagenomic sequencing of an unusual case of polymicrobial bacteremia. *J Infect* 2017;**75**(3):278–80.
2. Christin L., Sarosi G.A.. Pyomyositis in North America: case reports and review. *Clin Infect Dis* 1992;**15**(4):668–77.
3. Crum N.F.. Bacterial pyomyositis in the United States. *Am J Med* 2004;**117**(6):420–8.
4. Patel S.R., Olenginski T.P., Perruquet J.L., Harrington T.M.. Pyomyositis: clinical features and predisposing conditions. *J Rheumatol* 1997;**24**(9):1734–8.
5. Podglajen I., Bellery F., Poyart C., Coudol P., Buu-Hoï A., Bruneval P., et al. Comparative molecular and microbiologic diagnosis of bacterial endocarditis. *Emerg Infect Dis* 2003;**9**(12):1543–7.
6. Rampini S.K., Bloemberg G.V., Keller P.M., Büchler A.C., Dollenmaier G., Speck R.F., et al. Broad-range 16S rRNA gene polymerase chain reaction for diagnosis of culture-negative bacterial infections. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2011;**53**(12):1245–51.
7. Yang S., Rothman R.E.. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *Lancet Infect Dis* Jun 2004;**4**(6):337–48.

Thomas Gabas*
Assistance Publique-Hôpitaux de Paris, CHU de Bicêtre, Service de Maladies Infectieuses et Tropicales, Le Kremlin-Bicêtre, France Service de Maladies Infectieuses et Tropicales, Centre Hospitalier Sud Francilien, 40 avenue Serge Dassault, 91106 Corbeil Essonnes, France

Isabelle Podglajen
Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Microbiologie and Université Paris Descartes, Paris, France

Geoffrey Cheminet, Jean-Charles Gagnard, Benjamin Wyplosz
Assistance Publique-Hôpitaux de Paris, CHU de Bicêtre, Service de Maladies Infectieuses et Tropicales, Le Kremlin-Bicêtre, France

*Corresponding author at: Service de Maladies Infectieuses et Tropicales, Centre Hospitalier Sud Francilien, 40 avenue Serge Dassault, 91106 Corbeil Essonnes, France.
E-mail address: thomas.gabas@chsf.fr (T. Gabas)

Accepted 9 August 2019
Available online 17 August 2019

https://doi.org/10.1016/j.jinf.2019.08.008

© 2019 The British Infection Association. Published by Elsevier Ltd. All rights reserved.

Prenatal valacyclovir treatment of fetal citomegalovirus infection: A case series

Dear Editor,

We read with interest the article by Lim et al. published in June 2017 (Congenital cytomegalovirus - who, when, what-with and why to treat?).[1]

Citomegalovirus (CMV) is the most common congenital viral infection and is a leading cause of disability. Nowadays there are neither vaccines for primary prevention, neither approved therapies for the treatment of maternal-fetal infection. A French research group demonstrated that maternal use of high-dose Valacyclovir in symptomatic fetal CMV infection reaches therapeutic concentrations, reduces viral load in fetal blood and seems to reduce

Table 1
Details of maternal infection.

Case Number	Presumed week of infection (range)	Valacyclovir beginning (GE)	Time (wks) between presumed infection (mean) and initiation of therapy with Valacyclovir	Valacyclovir therapy stop (GE)	Weeks of therapy (n°)	GE at amniocentesis	CMV-PCR in amniotic fluid (copies/ml)	CMV amniotic fluid colture	US symptoms
1	8–16	23	11	40	17	21	3.175.940	Pos	no
2.1	4–9	22	15.5	35	13	22	56.000.000	Pos	IUGR, Hyperechogenic bowel
2.2	4–9	22	15.5	35	13	22	98.000.000	Pos	Hyperechogenic bowel
3	6–12	20	11	38	18	19	9.270	NP*	No
4	5–11	21	13	41	20	20	887.750	Pos	No
5	0–12	20	14	41	21	20	1.750.000	Pos	No
6	0–6	22	19	41	19	21	3.500	Neg	No
7	12–19	25	9,5	38	13	21	595	NP*	No
8	18–19	25	6.5	40	15	24	73.150	Pos	No
9	6–12	21	12	40	19	20	1.603.000	Pos	No
10	0–8	22	18	41	19	21	<42.5	Neg	No

NP* Not performed, amniocentesis performed in other center.
GE: Gestational Age.
Neg: Negative.
Pos: Positive.
US: Ultrasound scan symptoms.
IUGR: Intrauterin Growth Restriction.

substantially the rate of symptomatic infected infants compared with historic controls.[2,3] We describe a case series of Valacyclovir use in fetal asymptomatic CMV infection in women referred to our Prenatal Diagnosis Service (Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy). This study followed the protocol for off-label use applied in our Institution. Women with a diagnosis of primary CMV maternal infection in periconception period, first and second trimester of pregnancy referred to our center from August 2016 to October 2018 were evaluated. CMV serology, IgG Avidity molecular test for CMV of blood, molecular and cultural tests of urine and pharyngeal exudates for CMV were performed. Valacyclovir treatment was offered to women with a diagnosis of fetal CMV infection, defined as positivity of CMV-DNA detection by PCR or culture on amniotic fluid (AF). All patient gave written consent, after having understood the investigational nature of antiviral therapy. Valacyclovir was administrated per os at a dose of 8 g/die from the diagnosis of fetal infection until delivery. The first visit will be made 15 days after first Valacyclovir administration. Subsequent visits were programmed monthly. The blood count and liver/kidney maternal function tests, CMV serology, molecular test for CMV of blood, molecular and cultural tests of urine and pharyngeal exudates for CMV will be made 15 days after starting therapy and then every 30 days. Second level Ultrasound scan (US) will be made every thirty days. Timing and route of delivery were determined by standard maternal and fetal indications. In order to confirm diagnosis of infection in the newborn, anti-CMV IgM and CMV-DNA detection by PCR on cord blood was performed, and placentas were submitted for microbiologic examination. Within 3 weeks from birth, CMV-DNA detection by PCR and anti CMV IgM on neonatal blood and CMV-DNA detection by PCR on neonatal urine were performed. Clinical and neurological examination of the newborn, cranial circumference measurement, blood count, renal and hepatic function tests, cerebral US, TEOAES and fundus oculi examination were performed before discharge. Ten women were treated: 9 singleton pregnancies and a bichorionic biamniotic twin pregnancy (Table 1). Mean gestational age (GA) at amniocentesis was 20.9 (±1.37 SD). Valacyclovir therapy was started at a mean GA of 22.1 weeks (±1.79 SD) and continued until delivery. The drug was administrated for a mean of 17.4 weeks (±1.2.84 SD). No adverse events were reported. Mean GA at delivery was 39.18 (±2.36 SD). Mean birthweight was 2.650 g (±577.94 SD). Eight fetuses had positive virology for CMV infection at birth; only one was symptomatic at birth (case 2.2), while an infant developed a Bilateral Hypoacusia at 8 months of age (case 5) (Table 2). The symptomatic infection rate at birth was 1/11 (9%) and the rate of late sequelae was 9% (1/11). In the symptomatic infant (case 2.2), US scan revealed bowel hyperechogenicity after amniocentesis. Viral load on amniotic fluid was higher compared to the asymptomatic infected fetuses in this group. Furthermore, her mother was administered Valacyclovir for only 13 weeks. At birth she presented a Y-GT elevation in absence of other signs of visceral damage. Therefore, TEOAEs revealed a bilateral REFER result, and cerebral US showed a mild bilateral periventricular hyperechogenicity with mild ventriculomegaly. At sixth month audiometric examination revealed a profound bilateral hearing loss. The child underwent surgery for a bilateral cochlear implant. MRI performed at 14 months of age detected areas of hyper-intensity in the white matter in fronto-parietal region compatible with areas of delayed myelination. Currently, the child has a normal psychomotor development. Case 5 developed a sensorineural hearing loss at 8 months of life and started therapy with Valganciclovir. In these series, Valacyclovir was administered for a long time without side effects, demonstrating to be safe for mother and infants. We observed 3 newborns (case 6,7,10) who had negative serology and virology at birth, with an observed rate of negativity at birth of 27,3% (3/11). Moreover, they showed no clinical or instrumental sign of infection. In case 10, the number of copies in the amniotic fluid was low (<42,5 copies/ml) and a false positive cannot be excluded, although according unpublished data a false positive never occurred in our center. In cases 6 and 7 the number of copies of CMV on amniotic fluid was 3500 copies/ml and 595 copies/ml respectively. In two cases (6 and 10) AF colture was negative at 15 days, probably due to low viral load. Negative virology at birth, anyway, may represent an index of a favorable prognosis, considering that blood viral load is emerging as a marker to predict sequelae.[4–7] To the best of our knowledge, our series represent the first experience of high-dose Valacyclovir use in asymptomatic fetuses following a prenatal diagnosis of fetal CMV infection. The main limitation of these series is represented by low sample size and the lack of a control group. Therefore, we suppose that Valacyclovir could be effective especially in low AF viral load, reducing viral replication and allowing a microbiological clearance at birth. Low-viral load could be reached also with a prenatal treatment following diagnosis of maternal CMV infection. Starting high dose Valacyclovir follow-

Table 2
Neonatal outcome.

Patient number	CMV-PCR in amniotic fluid (copies)	Neonatal outcome	GE at birth	Mode of delivery	Weight at birth (grams)	IgG (s/co)	IgM (s/co)	Blood CMV-PCR (copies/ml)	Urine CMV-PCR (copies/ml)	Pharyngeal swab CMV-PCR (copies/ml)	Placental CMV-PCR	Placental Colture
1	3,175,940	Asymptomatic infected	40	VD	2.650	6.08	neg	596	16.480.384	pos	NA	Neg
2.1	56,000,000	Asymptomatic infected	35	CS	1.500	10.5	2.35 pos	10.000	>90.000.000	Pos	Pos	Neg
2.2	98,000,000	Symptomatic infected	35	CS	1.700	12.6	1.68 Pos	NA	85.000.000	Pos	Pos	Neg
3*	9270**	Asymptomatic infected	38	VD	3.100	71	Neg	2.490	Pos**	NA**	NA**	NA**
4	887,750	Asymptomatic infected	41	VD	2.630	12,1	Neg	259	>90.000.000	Pos	Pos	Neg
5	1,750,000	Postnatal Sequelae	41	VD	3.140	9,49	Neg	233	35.000.000	Pos	Pos	NA**
6	3500	Negative	41	VD	3.100	9,41	Neg	Neg	Neg	Neg	Neg	Neg
7*	595**	Negative	38	VD	2.415	>180**	Neg**	Neg**	Neg**	NA**	NA**	NA**
8*	73,150	Asymptomatic infected	40	VD	2.990	63,3**	Neg **	<122	8.855	NA	Pos	NA**
9	1,603,000	Asymptomatic infected	41	VD	2.735	4,53	Neg	49.987	165.550.000	Pos	NA	NA**
10	<42,5	Negative	41	CS	3.190	9,31	Neg	NA	Neg	Neg	Neg	NA**

GE: Gestational Age.
VD: Vaginal Delivery.
CS: Cesarean Section.
NA Not Available.
** Data from other center.
* Birth in other center.

ing maternal diagnosis of CMV infection could reduce vertical transmission or viral load in amniotic fluid, leading to a better prognosis for fetuses and infants. Although our results seem to be encouraging, randomized controlled trials are needed to demonstrate the role of Valacyclovir reducing vertical transmission and/or viral load in AF, infection and symptoms at birth and long-term sequelae.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Lim Y., Lyall H.. Congenital cytomegalovirus - who, when, what-with and why to treat? *J Infect.* 2017;**74**(Suppl 1):S89–94.
2. Leruez-Ville M., Ville Y.. Fetal cytomegalovirus infection. *Best Pract Res Clin Obstet Gynaecol* 2017;**38**:97–107.
3. Jacquemard F., Yamamoto M., Costa J.M., Romand S., Jaqz-Aigrain E., Dejean A., et al. Maternal administration of valaciclovir in symptomatic intrauterine cytomegalovirus infection. *BJOG* 2017;**114**(9):1113–21.
4. Lanari M., Lazzarotto T., Venturi V., Papa I., Gabrielli L., Guerra B., et al. Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. *Pediatrics* 2006;**117**:e76–83.
5. Boppana S.B., Fowler K.B., Pass R.F., Rivera L.B., Bradford R.D., Lakeman F.D., et al. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. *J Pediatr* 2005;**146**:817–23.
6. Forner G., Abate D., Mengoli C., Palù G., Gussetti N.. High cytomegalovirus (CMV) DNAemia predicts cmv sequelae in asymptomatic congenitally infected newborns born to women with primary infection during pregnancy. *J Infect Dis* 2015;**212**(1):67–71.
7. Bradford R.D., Cloud G., Lakeman A.D., Boppana S., Kimberlin D.W., Jacobs R., et al. Detection of cytomegalovirus (CMV) DNA by polymerase chain reaction is associated with hearing loss in newborns with symptomatic congenital CMV infection involving the central nervous system. *J Infect Dis* 2005;**191**:227–33.

Marco De Santis
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Ostetricia e Patologia Ostetrica, Dipartimento di Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Roma, Italy
Centro Studi per la Tutela della Salute della Madre e del Concepito, Università Cattolica del Sacro Cuore, Istituto di Clinica Ostetrica e Ginecologica, Roma, Italy

Carmen De Luca, Massimo Apicella, Luisa D'Oria
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Ostetricia e Patologia Ostetrica, Dipartimento di Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Roma, Italy
Università Cattolica del Sacro Cuore, Istituto di Clinica Ostetrica e Ginecologica, Roma, Italy

Antonio Lanzone
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Ostetricia e Patologia Ostetrica, Dipartimento di Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Roma, Italy
Centro Studi per la Tutela della Salute della Madre e del Concepito, Università Cattolica del Sacro Cuore, Istituto di Clinica Ostetrica e Ginecologica, Roma, Italy

Maurizio Sanguinetti
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Microbiologia, Dipartimento di Scienze di Laboratorio e Infettivologiche, Roma, Italy
Università Cattolica del Sacro Cuore, Istituto di Microbiologia, Roma, Italy

Piero Valentini
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Pediatria, Dipartimento di Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Roma, Italy
Università Cattolica del Sacro Cuore, Istituto di Clinica Pediatrica, Roma, Italy

Giovanni Scambia
Centro Studi per la Tutela della Salute della Madre e del Concepito, Università Cattolica del Sacro Cuore, Istituto di Clinica Ostetrica e Ginecologica, Roma, Italy
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Ginecologia Oncologica, Dipartimento di Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Roma, Italy

Lucia Masini[1]
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Ostetricia e Patologia Ostetrica, Dipartimento di Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Roma, Italy
Centro Studi per la Tutela della Salute della Madre e del Concepito, Università Cattolica del Sacro Cuore, Istituto di Clinica Ostetrica e Ginecologica, Roma, Italy

Rosaria Santangelo[1]
Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC di Microbiologia, Dipartimento di Scienze di Laboratorio e Infettivologiche, Roma, Italy
Università Cattolica del Sacro Cuore, Istituto di Microbiologia, Roma, Italy

*Corresponding author at: Dipartimento di Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Fondazione, Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, L.go A. Gemelli 8, Rome, Italy.
E-mail address: carmen.deluca@unicatt.it (C. De Luca)

[1] These authors contributed equally to this work.

Accepted 20 August 2019
Available online 26 August 2019

https://doi.org/10.1016/j.jinf.2019.08.015

© 2019 The British Infection Association. Published by Elsevier Ltd. All rights reserved.

Novel reassortant H7N2 originating from the H7N9 highly pathogenic avian influenza viruses in China, 2019

Dear Editor,

In recent years, evolution, reassortant, pathogenicity and antigenicity of H7N9 AIVs have been documented in this journal.[1–4] Since 2013, over 1400 cases of human infection with novel H7N9 influenza viruses have been reported in China, associated with a high rate of mortality.[5] Studies on circulating H7N9 influenza viruses have suggested origination from poultry.[6] However, strains isolated from live bird markets displayed low pathogenicity in poultry.[7] H7N9, a highly pathogenic avian influenza virus strain (HPAIV) has led to over 17 infection outbreaks in poultry in China since mid-2016.[8–10] To control infection of poultry by H7N9 and reduce the risk of human exposure, an inactivated H7 vaccine (H7-Re1) was developed and used nationally since September 2017, which successfully resulted in a significant decrease in the prevalence of H7N9 AIVs in both poultry and humans.[11,12] In December 2018, the original vaccine was replaced with H7-Re2 vaccine based on surveillance. In May 2019, an H7N2 AIV, A/chicken/China/SJZ1/2019 (SJZ1) was detected from H7–Re2-vaccinated layers. However, the pathogenicity and antigenicity of the H7N2 reassortant remain unclear. In this study, we present a brief assessment of the genetic and molecular characteristics, pathogenicity, and antigenicity of SJZ1.

PB2, PB1, PA, HA, M, and NS genes of SJZ1 were also closely related to those of H7N9 subtype viruses, such as A/chicken/Henan/SD163/2017(H7N9), which are circulating in China. NP and NA genes of SJZ1 were most closely related to those of A/chicken/Shanghai/10/2018(H9N2) and A/chicken/Fujian/SD070/2017(H9N2), respectively (Table S1). On the basis of analysis of phylogenetic relationships, we found that SJZ1 was a reassortant virus that derived its genes from a virus of a different subtype from poultry in China.

Based on the deduced amino acid sequence of HA, SJZ1 strain contained multiple basic amino acids (PKRKRTAR/GLF) at the cleavage site, suggestive of high pathogenicity. This theory was further confirmed by analysis of the intravenous pathogenicity index (IVPI). The strain was highly pathogenic in chickens, with IVPI value of 2.26, but showed low pathogenicity in ducks. No mortality or symptoms of infection were observed within 14 days post-inoculation (p.i.), although virus could replicate in the internal organs (brain, lungs, spleen, liver, intestine, and kidneys) of inoculated ducks on days 3 and 5 p.i. (Table S2).

The amino acid residues at the receptor binding site of HA protein were determined as Q226 and G228 (H3 numbering), suggesting that these viruses preferentially bind avian-like receptors.[13]

The phylogenetic tree based on the HA gene showed that SJZ1 strain belongs to the Highly pathogenic H7N9 clade but are clearly distinguishable from HP H7N9 viruses isolated in 2017 and 2018 (Fig. 1).

Amino acid identities of the HA gene segments of SJZ1 strain was 92.9% with H7-Re1 and 95.0% with H7-Re2. To evaluate the antigenicity and protective efficacy of the current H7-Re2 vaccine, SPF chickens were vaccinated with H7-Re2 and rSJZ1 (a reverse genetic recombinant carrying HA and NA of SJZ1 with internal gene segments of PR8).

Cross-reactive HI titers of H7-Re2 antiserum against SJZ1 virus were 6.5 $\log_2$ lower than that against the homologous H7-Re2 antigen. In contrast, cross-reactive HI titers of the antiserum against H7-Re2 antigens from rSJZ1 virus were not markedly different from those against the two homologous H7N9 viruses. These results indicated that the SJZ1 virus exhibited rapid antigenic drift and distinct antigenicity relative to the H7-Re2 vaccine strain.

During the 10-day observation period after challenge, H7-Re2-vaccinated birds displayed clinical signs of infection, such as depression, huddling, decreased feed and water consumption. Moreover, shed virus was detected in tracheal and cloacal swabs from all experimental chickens on 3 and 5 days post-challenge (p.c.), and only 10.0% of the challenged chickens survived, indicating a poor protective effect of the current H7-Re2 vaccine against SJZ1.

Notably, all rSJZ1-vaccinated birds survived with no clinical signs of infection. In addition, no virus shedding was detected in tracheal or cloacal swabs from any rSJZ1-vaccinated chickens on days 3 and 5 p.c. (Table 1). Interestingly, antiserum against the rSJZ1 virus showed a broader spectrum of reactivity to other viruses, including H7-Re2.

In this study, we reported the emergence of a new reassortant of SJZ1 which was generated by reassortment between the H7N9 AIVs and H9N2 AIVs in China. Results showed that SJZ1 exhibited rapid antigenic drift and distinct antigenicity relative to the H7-Re2 vaccine strain, which provides poor protection for SJZ1. It warns of the challenge we still face to control the H7N9 AIVs and their reassortants. Monitoring of the prevalence of not only the H7N9 AIVs but also reassortant H7 AIVs is essential to prevent the dissemination of these viruses.

Declaration of Competing Interest

None.

Acknowledgments

GDAS Special Project of Science and Technology Development (2019GDASYL-0302007).

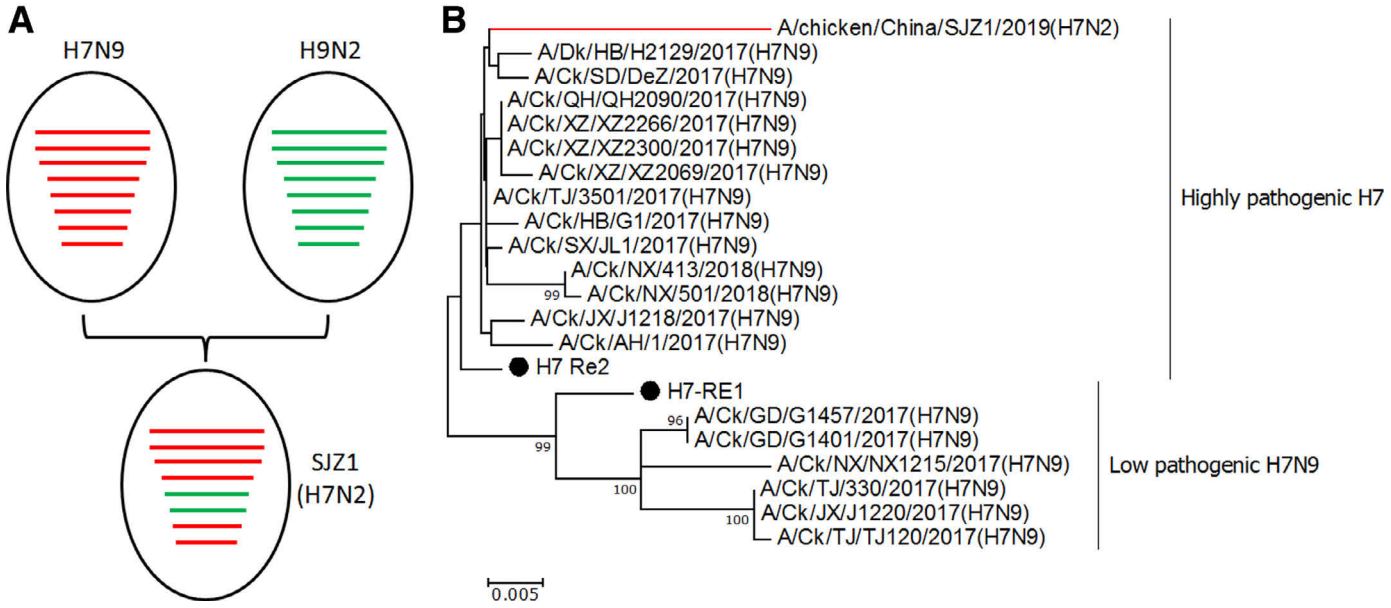

Fig. 1. Putative genomic compositions (A) and phylogenetic analysis of the hemagglutinin gene (B) of novel reassortant H7N2 virus isolated from poultry in China, 2019. Trees were constructed with MEGA6.05 software using the neighbor-joining method. Bootstrap analysis was performed with 1000 replications. "•" represents vaccine strains. Scale bars indicate the number of nucleotide substitutions per site.

Table 1
Efficacy of H7-Re2 vaccine against SJZ1 highly pathogenic avian influenza viruses in chickens.

Vaccine group	Challenge virus	Mean HI titer 21 days after immunization (log2)		Virus shedding				Survival
				Day 3 p.c.		Day 5 p.c.		
		Challenge virus	H7-Re2	Tracheal	Cloacal	Tracheal	Cloacal	
H7-Re2	SJZ1	1.7 ± 0.3	8.2 ± 0.4	6/6 (3.6 ± 0.4)	6/6 (4.4 ± 0.4)	3/3 (4.3 ± 0.4)	3/3 (4.5 ± 0.4)	1/10
rSJZ1	SJZ1	8.1 ± 0.5	6.6 ± 0.4	0/10	0/10	0/10	0/10	10/10
Control	SJZ1	< 1	< 1	4/4 (4.3 ± 0.5)	4/4 (4.6 ± 0.4)	NA	NA	0/10

[a] pc: post challenge.
[b] NA: not applicable due to death of chickens.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.08.016.

References

1. Pu Z., Luo T., Yang J., Shen X., Irwin D.M., Liao M., et al. Rapid evolving H7N9 avian influenza A viruses pose new challenge. *J. Infect* 2019;**78**(3):249–59 PubMed PMID:30367886.
2. He L., Liu D., Hu J., Shi L., Liu J., Cai T., et al. Two novel reassortant high pathogenic H7N9 viruses isolated in southern China in fifth wave shows internal genomic diversity and high virulence in chickens and ducks. *J Infect* 2018;**77**(6):561–71 PubMed PMID:30391631.
3. Hou G., Li J., Wang S., Cheng S., Peng C., Chen J., et al. Hemagglutinin characteristics, changes in pathogenicity, and antigenic variation of highly pathogenic H7N9 avian influenza viruses in China. *J Infect* 2019;**78**(2):158–69 PubMed PMID:30267798.
4. Liu D., Zhang Z., He L., Gao Z., Li J., Gu M., et al. Characteristics of the emerging chicken-origin highly pathogenic H7N9 viruses: a new threat to public health and poultry industry. *J Infect* 2018;**76**(2):217–20 PubMed PMID:28941628.
5. Su S., Gu M., Liu D., Cui J., Gao G.F., Zhou J., et al. Epidemiology, evolution, and pathogenesis of H7N9 influenza viruses in five epidemic waves since 2013 in China. *Trends Microbiol* 2017;**25**(9):713–28 PubMed PMID:28734617.
6. Gao R., Cao B., Hu Y., Feng Z., Wang D., Hu W., et al. Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med* 2013;**368**(20):1888–97 PubMed PMID:23577628.
7. Chen Y., Liang W., Yang S., Wu N., Gao H., Sheng J., et al. Human infections with the emerging avian influenza A H7N9 virus from wet market poultry: clinical analysis and characterisation of viral genome. *Lancet* 2013;**381**(9881):1916–25 PubMed PMID:23623390.
8. Wang N., Sun M., Wang W., Ouyang G., Chen Z., Zhang Y., et al. Avian influenza (H7N9) viruses co-circulating among chickens, southern China. *Emerg Infect Dis* 2017;**23**(12):2100–2 PubMed PMID:29148388Pubmed Central PMCID:5708235.
9. Yang L., Zhu W., Li X., Chen M., Wu J., Yu P., et al. Genesis and spread of newly emerged highly pathogenic H7N9 avian viruses in mainland China. *J Virol* 2017;**91**(23) PubMed PMID:28956760 Pubmed Central PMCID:5686710.
10. Shi J., Deng G., Kong H., Gu C., Ma S., Yin X., et al. H7N9 virulent mutants detected in chickens in China pose an increased threat to humans. *Cell Res* 2017;**27**(12):1409–21 PubMed PMID:29151586Pubmed Central PMCID:5717404.
11. Shi J., Deng G., Ma S., Zeng X., Yin X., Li M., et al. Rapid evolution of H7N9 highly pathogenic viruses that emerged in China in 2017. *Cell Host Microbe* 2018;**24**(4):558–68 e7 PubMed PMID:30269969 Pubmed Central PMCID:6310233.
12. Jiang W., Hou G., Li J., Peng C., Wang S., Liu S., et al. Prevalence of H7N9 subtype avian influenza viruses in poultry in China, 2013–2018. *Transbound Emerg Dis* 2019;**66**(4):1758–61 PubMed PMID:30903740.
13. Tharakaraman K., Raman R., Viswanathan K., Stebbins N.W., Jayaraman A., Krishnan A., et al. Structural determinants for naturally evolving H5N1 hemagglutinin to switch its receptor specificity. *Cell* 2013;**153**(7):1475–85 PubMed PMID:23746829 Pubmed Central PMCID: 3760228.

Yuan Qiu
Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou, China

Rongzhao Sun, Guangyu Hou, Xiaohui Yu, Yang Li, Jinping Li
Laboratory of Surveillance for Avian Diseases, China Animal Health and Epidemiology Center, No. 369 Nanjing Road, Qingdao 266032, China

Qiang Zhang, Fasheng Zou
Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou, China

Hualei Liu, Wenming Jiang*
Laboratory of Surveillance for Avian Diseases, China Animal Health and Epidemiology Center, No. 369 Nanjing Road, Qingdao 266032, China

*Corresponding author.
E-mail address: civcul@163.com (W. Jiang)

Accepted 23 August 2019
Available online 29 August 2019

https://doi.org/10.1016/j.jinf.2019.08.016

© 2019 The British Infection Association. Published by Elsevier Ltd. All rights reserved.

Disseminated cystic echinococcosis of Ferdinando II de' Medici, Grand Duke of Tuscany (1610–1670)

Dear Editor,

Ferdinando II de' Medici (1610–1670), eldest son of Cosimo II and Maria Maddalena of Austria, was Grand Duke of Tuscany from 1621 to 1670. His reign was one of the longest in the history of Tuscany, but it also marked the beginning of the Grand Duchy's economic decline. Ferdinando was a patron of science and friend of Galileo Galilei, supporting him even after the scientist was condemned by the Inquisition.

The historians attributed the death of Ferdinando, which occurred quite suddenly at the age of 60, to an ictus following dropsy.[1,2] However, a careful analysis of some documents from the rich archives of the Medici family allows to establish with reasonable certainty the exact cause of death. In particular, the necroscopic report written by the court surgeons, who carried out the autopsy on the body of Ferdinando two days after his death, contains relevant anatomo-pathological details. The court physicians, including the famous Francesco Redi (1626–1698), friend and confidant of the Grand Duke, reported that "The spleen [was] of a natural size and shape, a little pale, with two vesicles full of water. The membrane that entirely cover internally the thorax [i.e., the parietal pleura] was sprinkled with vesicles full of clear water, two of which [were] larger than the others and four fingers long […] and they broke slightly when pressed with the finger. […] The lungs, bad in color and consistency, were externally full of watery vesicles, which are called "hydatids" by the doctors […]. At the base of the heart, there were many vesicles of different sizes full of water gathered together like a bunch of grapes".[3]

The picture described by the doctors is a typical case of disseminated cystic echinococcosis, a parasitic disease caused by tapeworms *Echinococcus multilocularis*.[4] The diagnosis was clear even to the doctors, who defined the cysts as 'hydatids'. In fact, at the time of Ferdinando echinococcosis was a well-known condition. The first scholar who described the disease was Hippocrates, by describing it as a "fluid-filled liver", and then it was also recognized by Aretaeus, Galen and the Arabian Razes;[5] however, it was not until the past couple of hundred years that real progress was made in determining and describing its parasitic origin. It is noteworthy that the "animal" nature of the hydatid cysts of echinococcosis was illustrated just by Francesco Redi in 1684, a few years after the death of Ferdinando.[5] Only in 1863 *E. multilocularis* was identified by Leuckart and during the early to mid-1900s, the more distinct features of its life cycle and how it cause disease were fully described and studied.[6]

Fig. 1. Portrait of Ferdinando II de' Medici. Justus Sustermans, 1653–54 (Uffizi).

The disease is defined as 'zoonosis' since the definitive hosts are carnivorous predators, including dogs, wolves and foxes. The adult tapeworm lives in their small intestines and delivers eggs to be excreted with the excrement. The intermediate hosts are infected by ingesting eggs. Sheep, goats, cattle, camels, pigs, wild herbivores, and rodents are the usual intermediate hosts, but humans can also be infected as dead-end hosts.[4] Once ingested, the parasites hatches from the egg, penetrates the intestinal mucosa, and migrates through the bloodstream to internal organs such as the liver. A fluid-filled cyst (metacestode or hydatid cyst) develops in the affected organ after a period of time that can vary.[7]

The involvement of several thoracic organs in Ferdinando, in particular the heart, probably caused heart failure, which can well explain the dropsy described in the written sources, as well as the fatal outcome.

The documents related to ancient autopsies are a valuable source of information on the state of health of historical personages, and provide an indispensable contribution to the history of medicine (Fig. 1).

References

1. Acton H.. *The last medici.* London: Macmillan; 1962.
2. Lazzeri A.. *Il principe e il diplomatico. Ferdinando II tra il destino e la storia.* Firenze: Edizioni Medicea; 1996.

3. Pieraccini G.. *La stirpe dei medici di cafaggiolo*, vol. 2. Firenze: Nardini Editore; 1986.
4. McManus D.P., Zhang W., Li J., Bartley P.B.. Echinococcosis. *Lancet* 2003;**362**(9392):1295–304.
5. Eckert J.. Historische aspekte der echinococcose – einer uralten, aber noch immer relevanten zoonose. *Schweiz Arch Tierheilkd* 2007;**149**(1):5–14.
6. Leuckart R.. *Die Menschlichen Parasiten und die von Ihnen herruhrenden Krankheiten*, vol. 1. Lepzig u Heidelberg; 1863.
7. Agudelo Higuita N.I., Brunetti E., McCloskey C. Cystic echinococcosis. In: Kraft C.S., editor. *Cystic echinococcosis. J Clin Microbiol* 2016;**54.3**:518–23. *PMC*.

Raffaele Gaeta*
Valentina Giuffra
Division of Paleopathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Via Roma 57, 56126 Pisa, Italy

*Corresponding author.
E-mail address: raffaele.gaeta@med.unipi.it (R. Gaeta)

Accepted 23 August 2019
Available online 5 September 2019

https://doi.org/10.1016/j.jinf.2019.08.017

© 2019 The British Infection Association. Published by Elsevier Ltd. All rights reserved.