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Outbreak of adenovirus D8 in a neonatal intensive care unit involving multiple simultaneous transmission pathways

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SUMMARY

Background: Adenovirus (ADV) outbreaks in neonatal intensive care units (NICU) can lead to durable transmission and serious adverse outcomes. This study describes the investigation and control of an ADV-D8 outbreak in an NICU, associated with ophthalmologic equipment used during retinopathy of prematurity (ROP) screening. Cases were observed in neonates, parents and nurses.

Methods: The outbreak investigation was performed including sampling patients, parents and health care workers as well as the environment for molecular detection of ADV DNA. The investigation was also conducted in the guest house where some parents were temporary residents. A retrospective cohort study focused on neonates hospitalized during the epidemic period to assess the risk associated with ROP examination.

Results: Fifteen cases were identified in neonates; all but one presented with conjunctivitis. Two healthcare workers and 18 parents acquired conjunctivitis. ADV DNA was identified on the RetCam and on the freezer shared by parents. All ADV-positive samples were typed as ADV-D8. ADV infections occurred more frequently in neonates who had ROP examinations (37.8% (14/37) vs (0.9% (1/110); $P<0.001$) (relative risk 41.6; (5.7–305.8)). The RetCam was disinfected between two examinations using a disinfectant that was virucidal on ADV after a 30-min contact.

Conclusion: This outbreak was significantly associated with ROP examination with a RetCam that had a disinfection protocol ill-adapted to rapid patient turnover. In addition, nosocomial transmission via the parents to neonates and parent-to-parent transmission is

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likely to have played a role in the dissemination of cases. No further cases were observed after the new disinfection procedure was enforced.

Introduction

Human adenoviruses (ADVs) are non-enveloped resistant DNA viruses. Within the *Mastadenovirus* genus, there are seven species named *Human mastadenovirus A* to *G* totalling more than 70 genotypes. The existence of different organ tropisms explains differences in the clinical manifestations with ocular, respiratory or gastrointestinal symptoms. Strains belonging to *Human adenovirus B* and *D* species are the most common cause of infectious conjunctivitis and red eye worldwide, accounting for up to 75% of cases. They remain the first causative agent in clusters of viral conjunctivitis occurring in ophthalmology offices, clinics and hospitals. Adenoviruses 8, 19, 37 and 54 are the most prevalent genotypes involved in outbreaks of conjunctivitis [1].

Virus transmission occurs through hand to eye contact, ocular secretions, and respiratory droplets. Contagion is favoured by high viral loads commonly observed in patient secretions (several millions of virions/mL in a tear), and ADV ability to persist in the environment for up to 9 weeks increases the transmission [2]. Infection control in healthcare settings can therefore be challenging.

Although most infections are benign, immunocompromised patients can suffer from severe forms, sometimes fatal. In particular, ADV infections in neonatal intensive care units (NICUs) are associated with a mortality rate of 35% in the case of respiratory forms [3,4].

Most of the outbreaks are described in adult eye-care or ophthalmology departments. Epidemics are increasingly reported in NICUs with cases characterized by conjunctivitis or respiratory tract infections [5—13].

Thus study describes an outbreak of ADV ocular infections that occurred in one NICU of the University Hospitals of Marseille, France. Cases were described in preterm neonates, health care workers and parents. In addition, different transmission pathways were identified.

Methods

Overview of the neonatal centre

The level 3 neonatal centre (NNC) in this study is located in 'La Conception Hospital' (APHM University Hospitals of Marseille, France), and contains 54 beds of which 30 are grouped in an NICU and the remaining 24 beds are grouped in the neonatal medical unit (NNMU). The NICU consists of two single-bed rooms, eight two-bed rooms, and four three-bed rooms. In 2018, a total of 1138 patients were admitted to the NNC, of which 210 (19%) were high-level prematurity neonates (gestational age $<$32 weeks). The NNC is accessible to parents 24 h a day, 7 days a week. Individualized developmental care for babies and parents is the current practice in the NNC. Parent participation in neonatal care is encouraged and early, regular (daily) and prolonged skin to skin contact is promoted [14]. Retinopathy of prematurity is screened as recommended by the American Academy of Pediatrics and performed by RetCam [15].

A daily laboratory-based surveillance of healthcare-associated infections (HAI) has been implemented for years in the NNC. The infection control team participates in the weekly meeting during which catheter-associated bacteraemia rates and adverse care events have been monitored since 2005 and have been reported to the clinical team using bimonthly electronic news [11].

Epidemic investigation

The epidemic investigation consisted of (i) a detailed review of the medical record of case-patients, (ii) parent interviews, and (iii) an observational evaluation of the clinical practices. Information concerning dates of hospitalization, visits to the NNC, room number, demographic data, clinical signs associated with the ADV infection, accommodation of the parents hosted at the Ronald McDonald Foundation House (RMDFH). Active case finding included laboratory testing (detection of ADV DNA by real-time PCR assay) of each symptomatic patient and healthcare worker (HCW) in the NNC with respiratory, ocular or digestive symptoms. This was completed by a transversal screening of all patients hospitalized in the NICU on 7th June 2019. Parents were seen as outpatients, searched for conjunctivitis by the physicians of the NNC and samples were collected when possible.

An environmental survey was performed to identify potential sources of ADV through swab sampling on surfaces.

Case definition

A confirmed case in patients was defined by (i) clinical signs of conjunctivitis, keratoconjunctivitis, enterocolitis, gastroenteritis or respiratory symptoms AND (ii) presence of ADV DNA in conjunctival swab, or faecal or respiratory samples AND (iii) hospitalization in the NNC during the 14 days before the onset [2,12].

A confirmed case in HCWs was defined by (i) clinical signs of conjunctivitis or keratoconjunctivitis AND (ii) presence of ADV DNA in conjunctival swab AND (iii) the HCW had provided care to colonized or infected neonates.

A confirmed case in parents was defined by (i) clinical signs of conjunctivitis or keratoconjunctivitis AND (ii) presence of ADV DNA in conjunctival swab.

A probable case was defined by (i) clinical signs of conjunctivitis or keratoconjunctivitis AND (ii) the absence of clinical sample for which ADV DNA was tested AND (iii) hospitalization in the NNC during the 14 days before the onset [2,12].

Nomenclature for identification

The neonates were numbered according to the date of inclusion. The parents of the ADV-infected neonates were designated according to the child number. The letter (M or F)

designates the sex of the parent. The two parents who were identified as P*col*1M and P*col*2F are, respectively, the father (M) and the mother (F) of two neonates who were colonized (*col*) but not infected and therefore were not included in the group of 15 infected neonates.

Exposure to risk factors

A retrospective cohort study was performed among all preterm neonates hospitalized in the NICU and the NNMU from 29th April to 17th June 2019, to assess the risk of ADV infection after ROP examination. Attending ROP examination was analysed during the 14 days before the clinical onset of ADV infection or before the first detection of ADV DNA when sample was collected from a non-symptomatic patient. ROP examination was explored during the 14 days before ADV infection onset for each case-patient and during the exposed period for non-infected patients. A questionnaire form was used to collect information on infected parents and preterm neonates regarding date of onset, signs and symptoms of conjunctivitis.

Microbiological investigation

Clinical specimens (ocular swab, pharyngeal swab, faecal swab or stools) and environmental specimens were analysed at the virology laboratory of the university Hospitals of Marseille. Total nucleic acid extraction was performed using EZ-1 Advanced XL biorobot with the virus mini kit 2.0 (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Detection of ADV DNA was performed via real-time PCR using either the commercial Adenovirus R-gene kit (Argene, BioMerieux, Marcy l'Etoile, France) or the technique described by Heim *et al.* [16]. Positive samples were processed for determination of the ADV genotype either by PCR amplification and sequencing of the hexon hypervariable region as previously described [17], or by using six real-time species-specific PCR assays as previously described [18]. The latter was performed when hexon amplification failed due to low viral load.

Next-generation sequencing (NGS) was used to determine complete genome sequences of the virus detected in the ophthalmic swab of neonate N5 using the Ion-Torrent Platform with PGM (Life Technologies). Based on complete genome sequences available in Genbank database, a set of specific primers was designed using the 'Primal Scheme' programme (http://primal.zibraproject.org/) to amplify by PCR the complete viral genome. Pool of PCR products were sequenced following purification. Data analysis was performed using the CLC Genomics Workbench 6 software (CLC Bio).

Evaluation of the practices for patients

Usually, contact isolation precautions were systematically applied to each ADV-infected patient (i) either through transferring the patient in the unique single hospital room, (ii) or through cohorting in the two- or three-bed rooms when possible. The bedroom was disinfected daily using a quaternary ammonium solution (Surfanios). ROP examination was performed by the ophthalmologists without gloves for non-infected patients and with gloves for infected patients. Only single-use disposable blepharostats were used. After each ophthalmological examination, the disinfection of the lens, handle and keyboard of the RetCam was performed by the ophthalmologist using a wipe impregnated with didecyldimethylammonium chloride (Wip'Anios Excel, Anios, 59260 Lille-Hellemmes, France).

Statistical analysis

ROP-attending and ROP-unattending patients were compared for ADV infection using Fisher's exact test. Differences were considered significant at $P<0.05$. Statistical analyses were performed using PASW Statistics software version 17.0.

Ethics

This study was a description of the investigation of an epidemic conducted as part of hospital routine and the research department of the University Hospitals of Marseille confirmed that consultation of the ethics committee was not necessary.

Results

Cases

On 4th June 2019, the NNC reported a cluster of conjunctivitis among neonates and parents. From 14th May to 26th June 2019, 35 cases of ADV infection were identified: 15 cases were observed in hospitalized neonates (11 confirmed cases and four probable cases), 18 cases were observed in parents of those neonates, and two cases occurred in nurses working at the NICU (Figure 1). Among the 15 infected neonates, there were 14 conjunctivitis, 2 necrotizing enterocolitis (Bell stage 2A), 1 enteritis and 1 gastroenteritis [19]. Three neonates presented both conjunctivitis and digestive signs (Table I). Of them, N14 had a conjunctivitis that was laboratory-confirmed after he was transferred into the neonatal unit of another hospital (Table II).

All adult cases were seen in either HCWs or parents who had provided direct patient care or had direct contact with the infected or colonized neonates. Two nurses (H1 and H2), and six parents P9M, P12M, P15M, P15F, P*col*1M and P*col*2F presented with a keratoconjunctivitis, whereas other parents presented with conjunctivitis. Three parents were hosted at the Ronald McDonald Foundation House (RMDFH), a foundation building offering free temporary accommodation for parents of children hospitalized either in the NNC or in the oncology department. In 2018, the average length of stay of the parents was 17 days. Within families, conjunctivitis was transmitted to up to seven persons. Interestingly, conjunctivitis or keratoconjunctivitis among parents appeared during the second week of the outbreak, with four parents (P2F, P11F, P11M, P*col*2F) who were hosted in the RMDFH among the six first infected parents (Figure 1, Table II). Two parents, P4 and P8, developed conjunctivitis before their child became infected. All of the infected neonates and asymptomatic carriers had parents with conjunctivitis.

Epidemic investigation

During the same period, 147 neonates had been hospitalized in the NICU or in the NNMU. The attack rate in neonates was 10.2% (15/147). ADV infection occurred more frequently in neonates who had ROP examinations (14/37, [37.8%]) compared

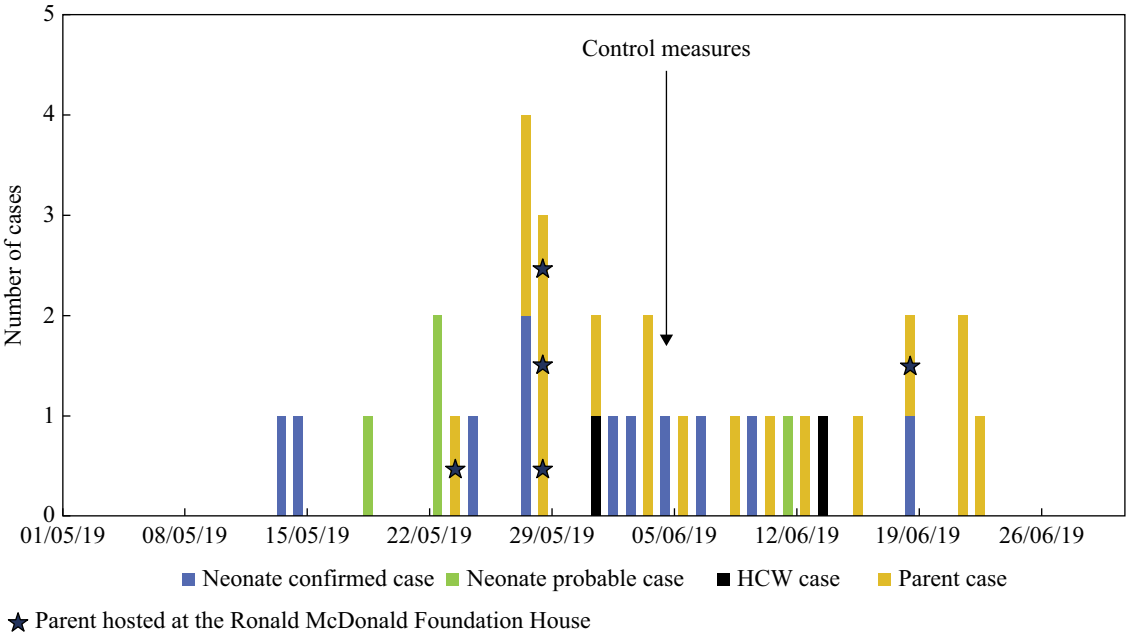

Figure 1. Epidemic curve. HCW, healthcare worker.

Table I
Demographic and clinical characteristics of the 15 neonate cases

Clinical characteristics	Data
Age at time of clinical signs (weeks), median (SD)	9 (6–17)
Gestational age (weeks)	
<30, *N*	11 (24–29)
≥30, *N*	4 (30–38)
Birth weight (g), median (SD)	910 (610–2270)
Gender	
Male, *N*	10
Female, *N*	5
Underlying comorbidity	
ROP, *N*	5
Bronchopulmonary dysplasia	8
Chronic digestive disturbance	1
Eye examination, *N*	
1	12
2	2
Time from first eye examination to clinical signs (days)	6 (1–14)
Time from second eye examination to clinical signs (days)	7–14

SD, standard deviation.

with neonates without ROP examination (1/110, [0.9%]): the difference was statistically significant ($P<0.001$) with a relative risk (RR) of 41.6 (95% confidence interval (CI), 5.7–305.8). The transversal 'one-day' screening of all patients in NICU bedrooms identified three additional neonates who were colonized (not infected). Due to low viral load, ADV typing was not possible but identification of *human Mastadenovirus D* species through specific real-time PCR was possible for two patients. It can be assumed that these two cases were infected with ADV-D8. A total of 12 environmental samples were collected on swabs and processed by the laboratory to identify potential sources of ADV. Of the nine swab samples collected on the RetCam, six were positive for ADV DNA, two on the handle, one on the lens, three on the keyboard. Of the three swab samples collected at the RMDFH on the handle of the plate cupboard, the buttons of the washing machines and the handle of the freezer on 28th June 2019, only the latter was positive for ADV DNA.

Microbiological investigation

From 10th May to 30th June 2019, the virology laboratory received 110 samples from patients hospitalized in the NNC and from HCWs, parents or the environment. The first ADV-positive PCR result was obtained from N1 on 14th May 2019, in a nasopharyngeal aspiration. During the outbreak, 11 neonates had at least one sample that tested positive for ADV DNA. ADV-D8 was identified in 10 neonates (N1 to N10) whereas N11 was not typed because it was found to be ADV DNA positive after having been transferred to another hospital.

During the outbreak period, there was no detection of ADV-D or ADV-D8 in patients hospitalized in other wards and HCWs of the University Hospitals of Marseille (four geographic sites).

Two nurses were found to be positive for ADV DNA on eye swabs collected because of clinical keratoconjunctivitis (both ADV-D of which H1 was typed as ADV-D8). Among the 18 parents who reported clinical signs of either conjunctivitis or keratoconjunctivitis, six were sampled and tested positive for ADV-D, of which five were typed as ADV-D8.

ADV DNA was detected in samples collected on the handle and the keyboard of the RetCam (Figure 2) and from the handle of a freezer in the RMDFH. This freezer had been used by four ADV DNA positive parents (P5/6F, P11F, P11M, P*col*2F) corresponding to three families hosted in the RMDFH. All the ADV-positive samples from the neonates, the HCWs, the parents

Table II
Characteristics of human cases and environmental samples

Category	Source	Sex	onset	sample collect	Sample type	ADV PCR result (1)	ADV species, type (2)	Infection	Case
Neonates	N1	F	14/05/2019	14/05/2019	bronchial aspiration	Ct 23	D8	conjunctivitis	confirmed
	N2	M	13/05/2019	NP (3)	NP	NP	NP	conjunctivitis	probable
	N2	M	23/05/2019	23/05/2019	stool	1.39 x 10e5 cp/mL	D8	gastroenteritis	confirmed
	N3	F	27/05/2019	28/05/2019	nostril swab	Ct 26	D8	conjunctivitis	confirmed
	N4	M	03/06/2019	04/06/2019	ophthalmic swab	5.52 x 10e6 cp/mL	D8	conjunctivitis	confirmed
	N4	M	27/05/2019	07/06/2019	Fecal swab	Ct 29	D8	necrotizing enterocolitis	confirmed
	N5	F	02/06/2019	04/06/2019	Stool	Ct 33	D8	necrotizing enterocolitis	confirmed
	N5	F	06/06/2019	06/06/2019	ophthalmic swab	4.01 x 10e8 cp/mL	D8	conjunctivitis	confirmed
	N6	M	04/06/2019	05/06/2019	ophthalmic swab	1.10 x 10e8 cp/mL	D8	conjunctivitis	confirmed
	N6	M	-	07/06/2019	Fecal swab	Ct 29	D	asymptomatic	confirmed
	N7	F	24/05/2019	07/06/2019	Fecal swab	Ct 36	D8	gastroenteritis	confirmed
	N8	M	06/06/2019	07/06/2019	ophthalmic swab	Ct 30	D8	conjunctivitis	confirmed
	N9	F	01/06/2019	13/06/2019	ophthalmic swab	Ct 28	D8	conjunctivitis	confirmed
	N10	M	18/06/2019	18/06/2019	ophthalmic swab	2.72 x 10e7 cp/mL	D8	conjunctivitis	confirmed
	N11	M	09/06/2019	19/06/2019	Nostril swab	positive (4)	NP	conjunctivitis	confirmed
	N12	M	18/05/2019	NP	NP	NP	NP	conjunctivitis	probable
	N13	M	22/05/2019	NP	NP	NP	NP	conjunctivitis	probable
	N14	M	22/05/2019	NP	NP	NP	NP	conjunctivitis	probable
	N15	M	11/06/2019	NP	NP	NP	NP	conjunctivitis	probable
HCWs	H1	F	13/06/2019	13/06/2019	ophthalmic swab	8.87 x 10e4 cp/mL	D8	kerato-conjunctivitis	confirmed
	H2	F	11/06/2019	11/06/2019	ophthalmic swab	3.15 x 10e3 cp/mL	D	kerato-conjunctivitis	confirmed
Parents	P3	M	10/06/2019	13/06/2019	ophthalmic swab	2.04 x 10e5 cp/mL	D8	conjunctivitis	confirmed
	P3	F	03/06/2019	13/06/2019	ophthalmic swab	Ct 36	D	conjunctivitis	confirmed
	P5/6 (5)	F	18/06/2019	19/06/2019	ophthalmic swab	2.39 x 10e6 cp/mL	D8	conjunctivitis	confirmed
	Pcol1 (6)	M	21/06/2019	21/06/2019	ophthalmic swab	Ct 26	D8	kerato-conjunctivitis	confirmed
	P15	F	22/06/2019	25/06/2019	ophthalmic swab	5.68 x 10e5 cp/mL	D8	kerato-conjunctivitis	confirmed
	P15	M	23/06/2019	26/06/2019	ophthalmic swab	2.31 x 10e5 cp/mL	D8	kerato-conjunctivitis	confirmed
	P2	F	23/05/2019	NP	NP	NP	NP	conjonctivitis	probable
	P4	M	27/05/2019	NP	NP	NP	NP	conjunctivitis	probable
	Pcol2	F	28/05/2019	NP	NP	NP	NP	kerato-conjunctivitis	probable
	P8	F	03/06/2019	NP	NP	NP	NP	conjonctivitis	probable
	P9	M	12/06/2019	NP	NP	NP	NP	conjunctivitis	probable
	P9	F	08/06/2019	NP	NP	NP	NP	kerato-conjunctivitis	probable
	P11	M	28/05/2019	NP	NP	NP	NP	conjunctivitis	probable
	P11	F	28/05/2019	NP	NP	NP	NP	conjunctivitis	probable
	P12	M	03/06/2019	NP	NP	NP	NP	kerato-conjunctivitis	probable
	P13	F	15/06/2019	NP	NP	NP	NP	conjunctivitis	probable
	P14	F	27/05/2019	NP	NP	NP	NP	conjunctivitis	probable
	Pcol1	F	31/05/2019	NP	NP	NP	NP	conjunctivitis	probable
Environment	E1	-	-	21/06/2019	retcam handle	1.28 x 10e3 cp/mL	NP	-	-
	E2	-	-	24/06/2019	retcam handle	1.07 x 10e3 cp/mL	D	-	-
	E3	-	-	28/06/2019	retcam handle	negative	NP	-	-
	E4	-	-	21/06/2019	retcam lens	negative	NP	-	-
	E5	-	-	24/06/2019	retcam lens	negative	NP	-	-
	E6	-	-	28/06/2019	retcam lens	negative	NP	-	-
	E7	-	-	21/06/2019	retcam keyboard	Ct 39	NP	-	-
	E8	-	-	24/06/2019	retcam keyboard	Ct 38	NP	-	-
	E9	-	-	28/06/2019	retcam keyboard	Ct 38	D	-	-
	E10	-	-	28/06/2019	RMDFH (7) washing machine	negative	NP	-	-
	E11	-	-	28/06/2019	RMDFH cupboard door	negative	NP	-	-
	E12	-	-	28/06/2019	RMDFH freezer handle	Ct 40	NP	-	-

(1) ADV PCR Result either from R-gene assay (cp/mL) or in-house qualitative assay derived from Heim et al (Ct value); (2) ADV species and type, D8 determined by sequencing and D determined by species specific real-time PCR
(3) Not Performed; (4) sample tested in a private medical laboratory suing a qualitative assay; (5) P5/6, twin neonates; (6) infected parent of a colonized neonate; (7) Ronald Mc Donald Fondation House

ADV, adenovirus; NP, not performed; QAPCR, polymerase chain reaction; RMDFH, Ronald McDonald Foundation House.

or the environment were typed as either D8 or D (Table II). Analysis of the complete genome of the virus present in the ophthalmic swab of newborn N5 confirmed that it was a D8 genotype.

Infection control measures

A total of 10 measures were implemented. (1) All neonate cases were subjected to the contact precautions protocol (CPP). (2) The CPP was supplemented with disinfection of the environment using 5% hypochlorite solution for each patient room, for the parents' kitchen (a room in the NNC ward where parents can relax and have coffee or fresh drinks), and for common staff areas in the NNC. The same disinfection protocol was implemented in the parent bedrooms, in the kitchen and in the laundry of the RMDFH. (3) Because of reported prolonged shedding of ADV [20,21], CPP was maintained until all neonate cases were discharged even if ADV PCR was negative. (4) NICU and NNMU personnel were screened for clinical signs of conjunctivitis and sampled for ADV PCR testing if symptomatic; those who were positive were banned from work for at least 14 days after onset. (5) A negative ADV PCR was required before staff reintegration. (6) Since 11th June 2019, symptomatic parents have been identified by the NNC staff, sampled, tested for ADV DNA and recommended not to visit for 14 days if positive. (7) Repeated training on hand hygiene, barrier precautions and disinfection of equipment was provided by the infection control team to the ophthalmology medical team and the HCWs. (8) Glove wearing was recommended for eye examination on infected neonates. (9) The parents who had contact with the neonates received education about hand hygiene and contact precautions. (10) The procedure for the disinfection of the RetCam after each patient was modified on 12th June 2019: the RetCam was cleaned and disinfected with 200 ppm chlorine dioxide wipes (Tristel Duo OPH, France); after each disinfection, the RetCam lens was rinsed with sterile water.

Discussion

This study describes a large outbreak of ADV-D8 infections in the NNC affecting neonates, HCWs and visiting parents. As soon

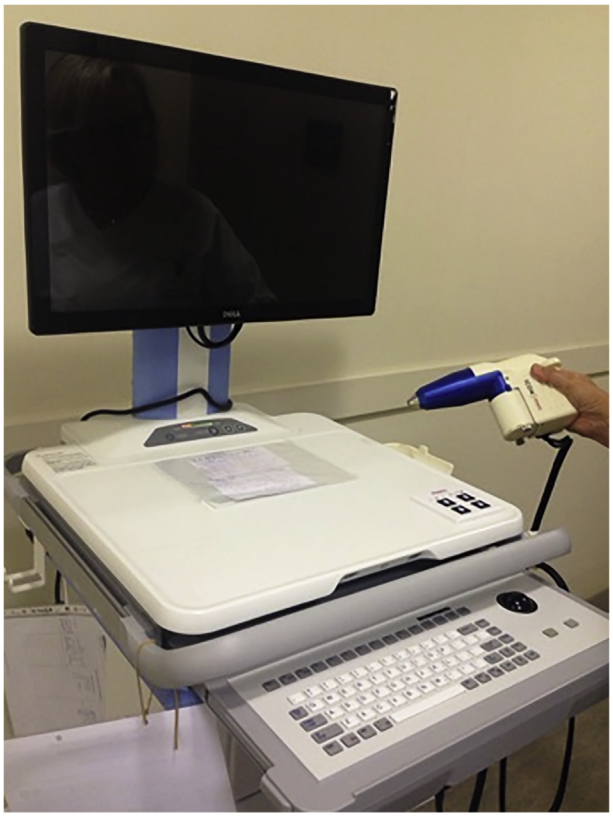

Figure 2. Handle and keyboard of the RetCam on which adenovirus (ADV) DNA was detected.

as the alert was launched by the virology laboratory, the infection control team visited the NNC, reviewed the procedures and proposed corrective actions to mitigate the epidemic situation and to define the source of infection as well as the pathways of transmission. It appeared that patient-to-patient transmission has occurred indirectly during ROP examination due to contamination and insufficient disinfection of this equipment.

ROP examination has been previously associated with ADV conjunctivitis in the NICU with respective odds ratio at 17.5 (95% CI = 1.9—163.0) and 84.6 (95% CI = 4.5—1601) [7,8]. In our study, ROP examination was associated with an RR of 41.6 (95% CI = 5.7—305.8) among neonates. Although most of the NICU outbreaks reported exclusively conjunctivitis cases, three outbreaks (caused by ADV-3, ADV-8 and ADV-30) described a combination of conjunctivitis and respiratory infections [4—6]. In our study the predominance of the ocular manifestations is indicative of the source of the infection. As previously described [8], all neonates presented with common conjunctivitis (no keratitis) that was initially believed to be bacterial conjunctivitis that is common in the NICU; this is likely to explain why clinical samples were collected and sent to the laboratory 9 days after the index case. Digestive manifestations were described in four neonates [22] of which three also had ocular manifestations. Surprisingly, neither neonates nor parents or HCWs presented with respiratory infections, even neonates who had pre-existing chronic lung disease.

In most NICU outbreaks, transmission has been reported via infected neonates [4,5,7,8]. Indirect transmission was mediated by ADV-contaminated ocular instruments and ophthalmic solutions, or through fomites, or hands of the NICU staff. In one study, an infected ophthalmologist was the possible source of an outbreak [4].

Our investigation showed that four parents presented a conjunctivitis early in the outbreak, and that two parents were infected before their child became infected. This suggests the possible involvement of infected parents as an additional source of contamination. Contact between parents and their neonates and skin to skin contact may have been a possible mode of transmission. Moreover, four parents were hosted at the RMDFH and shared the same freezer: because ADV DNA was detected on the handle of the freezer, transmission may have occurred among parents living at RMDFH. During the NICU outbreak period, there was no detection of ADV-D or ADV-D8 in patients hospitalized in other wards in the University Hospitals of Marseille (3400 beds, four geographic sites): this is of particular importance because the RMDFH was also hosting parents of children hospitalized in oncology units of the La Timone Children's Hospital, within walking distance of the NICU. The fact that there was no dissemination or transmission of ADV-D or ADV-D8 beyond the NICU is reassuring and suggests that the measures deployed in the NICU and at the RMDFH to mitigate the outbreak were efficient in preventing uncontrolled transmission. It also demonstrates the importance of performing environmental sampling in order to delineate risks of transmission.

This suggest that ADV was circulating among the neonates before it was transmitted to the parents and HCWs with secondary transmission between parents and adults to neonates. Interestingly, the two nurses were infected at the latest stage of the outbreak.

The detection of ADV DNA together with the presence of infected parents at RMDFH demonstrates that ADV circulation was potentially amplified by transmission pathways other that RetCam examination: accordingly, neonate-to-adult, adult-to-adult, and environment-to-human transmission pathways are plausible. Although the primary source remains the RetCam procedure, it is likely that other transmission routes occurred during this epidemic. The high environmental resistance of ADV was probably also a factor.

The RetCam is an ophthalmic equipment that comes into contact with mucous membranes and is therefore considered to be a semi-critical medical device according to Spaulding classification [23]. It is used commonly in NNMU in order to detect ROP. When investigating this outbreak, the infection control team identified that the disinfection of the RetCam (lens and surface) was inappropriate; it was carried out by using a wipe impregnated with quaternary ammonium solution that is virucidal on ADV after a 30-min contact. The time-contact was not respected between two patients. Hypochlorite (5000 ppm chlorine) and 70% ethyl alcohol for 5—10 min have also been found to be effective against ADVs [23] and are recommended for tonometer tip disinfection [24,25]. It is recommended that the RetCam be wiped clean and disinfected with a dioxide chlorine wipe that proves virucidal on ADV after 30-s contact. This procedure is also amenable for an efficient disinfection without disrupting the turnover use of RetCam. As

ROP examination was carried out on very preterm neonates, the potential adverse health outcome of chlorine on the eyes must be considered since because chemicals could injure the cornea. The RetCam disinfection was therefore completed by rinsing the lens and surface with a sterile wipe impregnated with sterile water. After implementation of our procedure, no additional case was observed.

Analysis of the complete genome sequence of epidemic ADV-D8 from the ocular sample of a neonate confirmed that it is the D8 genotype. Since 2019, epidemiological data from the virology laboratory of University Hospitals of Marseille have shown sporadic detection of this genotype in ophthalmic specimens, but secondary cases or epidemic features were not recorded. ADV-B3, ADV-B7 and ADV-D37, but not ADV-D8, have also been occasionally detected in eye samples from July 2019 to January 2023 (data not shown).

The investigation of this outbreak required a strong collaboration between the infection control team and the NNMU and NICU. It was performed in these two units and enlarged to the foundation where parents were hosted. Multiple prevention measures were successfully implemented to control the potential sources of contamination among patients, parents, HCWs, surfaces and medical devices. However neonates were not recalled after discharge to identify ADV infection and some infected patients may have been missed, which underestimated the number of cases. The source of contamination by adults was incompletely evaluated as asymptomatic carriers among parents and HCWs had not been screened. ROP examination was associated with ADV conjunctivitis in our study (RR = 41.6) with a large confidence interval (95% CI = 5.7—305.8) in relation to the small number of cases. Although most cases in babies were seen after ROP, other pathways of transmission have participated in the magnitude and the duration of the epidemic.

The originality of this ADV-D8 outbreak lies in the likelihood that multiple modes of transmission co-existed. Nosocomial transmission via direct contact with RetCam was significantly identified (RR = 41.6). Nosocomial transmission via the parents to neonates, and parent-to-parent transmission through direct contact or indirectly via contaminated kitchen appliances in the guest house may have also occurred. Interestingly no further cases were observed after the new disinfection procedure was enforced. In addition, enhanced surveillance and control in this department has prevented the occurrence of other ADV outbreaks since 2020.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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