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The newborns Torque teno virus dynamics depending on the term, feeding type and maternal viral load

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Summary. – The first weeks of life are extremely important for the development of the immunity-virome interaction that affects human health in adulthood. In this study we analyzed Torque teno virus (TTV) dynamics during the first weeks of life in the full-term/premature infants in relation with the maternal TTV load and the type of feeding. 152 infants aged 1–14 weeks (63 full-term and 89 premature) and 33 mother-child pairs were analyzed for the whole blood TTV load by qPCR with test sensitivity of 1000 viral copies/ml. 50 infants were retested (at 2–11 time points) for TTV dynamics data. All one-week babies (n = 71) from TTV-positive mothers were TTV-negative, consistently with the previous findings of the lack of transplacental transmission of the virus. TTV was not detectable in newborns under two weeks of age. Most infants are TTV-positive by 14 weeks of age. Whole blood TTV load does not show significant correlation with full-term/prematurity, maternal TTV load, or feeding type.

Keywords: Torque teno virus; transfusion-transmitted virus; commensal virus; TTV; viral load dynamics; TORCH infections; full-term and premature babies; breastfeeding; virome

The human microbiota is involved in a number of physiological processes vital to the host, such as the formation of the immune system, metabolism and energy, the maturation of intestinal epithelium, and neurobehavioral development and maintenance of tissue homeostasis (Yadav *et al.,* 2018). Healthy people have a very diverse microbiota (Heiman and Greenway, 2016), while various pathologies, including inflammatory diseases, diabetes, obesity, and cardiovascular disease, are associated with altered microbiota composition and decreased diversity (Turnbaugh *et al.,* 2006; Murri *et al.,* 2013; Machiels *et al.,* 2014; Romano *et al.,* 2015). Colonization by gut flora is critical to the growth and development of an infant and is highly dependent on genetics, mode of delivery, dietary patterns, maternal flora, and maternal age and lifestyle (Dominguez-Bello *et al.,* 2010; Rodríguez *et al.,* 2015).

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Abbreviations: TTV = Torque teno virus

Less is known about the effect of the viral population (virome), consisting of bacteriophages and eukaryotic RNA and DNA viruses, on pregnancy and early postnatal development. Wylie *et al.* (2018) showed that a high diversity of the vaginal eukaryotic DNA virome is associated with preterm delivery. Wolsk *et al.* (2016) suggested that the asymptomatic presence of picornavirus in the respiratory tract of newborns may act as a potent activator of the local immune response.

Microbiome of young infants is highly dynamic and subject to age-related changes in the composition of bacteria, eukaryotic viruses and bacteriophages (Lim *et al.,* 2015). Virome dynamics were studied in 20 pairs of Malawian twins aged 0 to 3 years with severe acute malnutrition or healthy; results showed that anelloviruses and circoviruses were differently represented in twins with varying degrees of malnutrition (Reyes *et al.,* 2015). It is known that the mode of delivery has a significant effect on the intestinal viral communities, since naturally born babies have a wide variety of eukaryotic viruses and bacteriophages. In particular, Torque teno virus (TTV)

was significantly enriched in the feces of naturally born infants independently of the breastfeeding status (McCann *et al.,* 2018).

The early postpartum period is critical for the formation and development of the immune system; it is vital to human health in adulthood (Lim *et al.,* 2015). Many factors predispose premature babies to the highest risk of pathological infections compared to all other age groups. The underdeveloped state of immune system, characteristic of prematurity, increases the risks of infection. Suppression of the innate immune system is prudent for development because it allows the fetus not only to safely be exposed to maternal antigens, but also to deal with physiological stress and remodeling. However, this makes the newborn, especially the premature one, very susceptible to bacterial and viral infections (Simon *et al.,* 2015).

The list of major viruses in newborns includes respiratory viruses (rhinovirus, picornavirus, parainfluenza viruses, respiratory syncytial virus, metapneumovirus, parachoviruses, coronavirus, enterovirus, adenovirus, bocavirus 1) and herpes viruses (cytomegalovirus, herpes simplex virus, herpes simplex virus) (Freij and Sever, 1988; James and Kimberlin, 2015; Luoto *et al.,* 2016; Pinninti and Kimberlin, 2018). Some of them persist in the fetus and newborn in a hidden way, without causing cytological abnormalities or detectable forms of the virus. Such latent infection can be associated with fetal and newborn disease, organ damage, and other complications (Robb *et al.,* 1986). The study of the neonatal virome and its relationship with the immune system is gaining in importance.

In this context, the study of the proliferation of the commensal TTV in the early postnatal period can provide important indicators of the state of immunity (Griffiths, 1999; Simmonds *et al.,* 1999; Mushahwar, 2000; Bendinelli *et al.,* 2001; Griffin *et al.,* 2008). TTV, which is ubiquitous in human body fluids and tissues, is associated with a state of immunosuppression in the early period after solid organ transplantation (Fernández-Ruiz *et al.,* 2019; Ruiz *et al.,* 2019). Data on intrauterine transplacental transmission of TTV vary, but recent PCR studies of umbilical cord blood show no virus in newborns (Tyschik *et al.,* 2017). TTV dynamics during the first year of life shows a significant positive correlation of viral load with age; the rapid growth during the first two months of life reaches plateau at 3–6 months (Tyschik *et al.,* 2018). Understanding the kinetics of primary TTV infection and its role in establishing chronic infection is important for determining early viral pathogenesis mechanisms or the possible association of TTV with immunity.

The aim of this study was to determine the TTV viral load in term and premature infants, taking into account the feeding type and maternal viral load.

The study protocol was reviewed and approved by the Ethics Committee of the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology (Protocol No. 2018/07.02); the study was conducted in accordance with the Declaration of Helsinki. All participants (parents) provided written informed consent.

This prospective, single-center study included 152 children (63 full-term and 89 premature, 0–14 weeks of age). Of the total 152 infants, 50 were tested repeatedly (at 2–11 time points), and 33 were included in mother-child pairs (with mother's analysis on TTV). All full-term infants were breastfed immediately from birth, and all premature infants were fed with formula throughout the period of blood collection. The exclusion criteria were as follows: signs of disorder (infectious or genetic), immunological deviations as assessed from blood counts, age over 4 months, voluntary refusal.

Each sample included two aliquots of capillary blood collected into Microvette 200 EDTA K3 tubes (Sarstedt, Germany) between February 2018 and June 2019 at the Kulakov National Medical Research Center for Obstetrics, Gynecology, and Perinatology (Moscow, Russia). DNA extraction, TTV quantification and qPCR data analysis were performed as described in (Tyschik *et al.,* 2018).

All one-week babies (n = 71) from TTV-positive mothers were TTV-negative, consistently with the previous findings of the lack of transplacental transmission of the virus (Fig. 1) (Tyschik *et al.,* 2017). Only two of the infants were TTV-positive before 4 weeks of age (with the test sensiti-

Table 1. The age of first TTV detection in preterm and full-term babies

	Age, days	TTV, copies/ ml
premature babies	13	$2{,}2 * 10^3$
	31	$1{,}5 * 10^4$
	42	$4{,}3 * 10^4$
	56	$4{,}6 * 10^3$
	76	$1{,}5 * 10^5$
	89	$9{,}8 * 10^4$
	89	$5{,}2 * 10^5$
	96	$5{,}7 * 10^3$
	105	$1{,}2 * 10^5$
full-term babies	42	$1{,}8 * 10^3$
	45	$1{,}5 * 10^4$
	64	$2{,}7 * 10^3$
	67	$8{,}4 * 10^5$
	111	$2{,}6 * 10^4$
	114	$4{,}0 * 1^{0}4$
	119	$3{,}2 * 10^5$

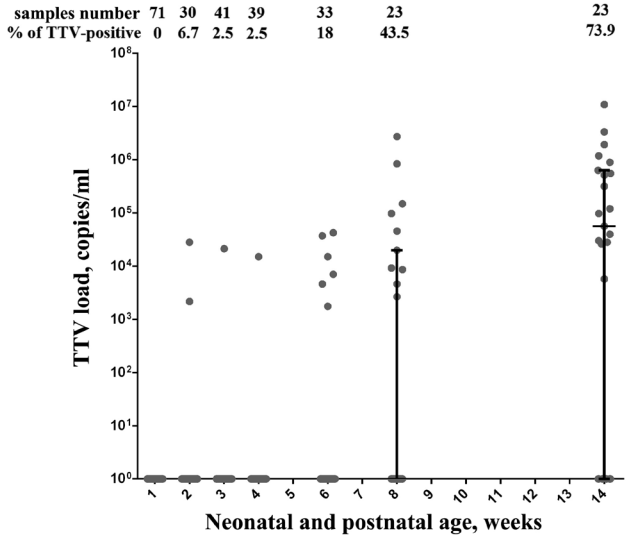

Fig. 1

TTV dynamics during the first weeks of life
The numbers above each group represent the total number of samples tested and the percent of TTV-positive samples.

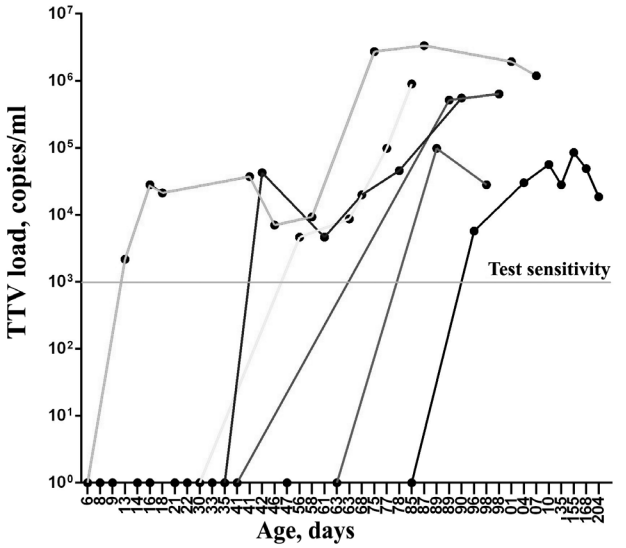

Fig. 2

Individual TTV dynamics for six TTV-positive infants with multiple blood sampling time points

vity of 1000 copies/ml (Vasilyev *et al.*, 2009)). The percent of TTV-positive samples increased from 18% at 6 weeks to 74% at 14 weeks of age. Time points of the earliest TTV detection in whole blood of preterm and full-term babies are given in Table 1.

Individual TTV dynamics for 6 children for whom the virus was detected in at least 2 blood samples over at least 3 time points is shown in Fig. 2.

Of the 33 examined mother-child pairs, 11 mothers (33.3%) were TTV-negative (with no positive infants (mean age 52 days)) and 22 mothers (66.7%) were TTV-positive (with 17 TTV-negative (mean age 53 days) and 5 TTV-positive infants (mean age 57 days) (Fig. 3). Viral load analysis of groups of TTV-positive mothers with TTV-negative and TTV-positive infants did not show significant differences in TTV load of mothers' whole blood (Mann-Whitney test, $p < 0.05$).

These data are consistent with the results of our previous study (Tyschik *et al.*, 2018) showing that TTV load increases during the first months of life in healthy children with strong proliferation during the first two months after birth. In publication of Ohto *et al.* (2002), a lower incidence of infection was also observed at the earliest age, and with age of 6 months and 2 years, a subsequent increase in the prevalence of TTV was revealed.

Some other authors also point rather to the absence of transplacental transmission of TTV (Fang *et al.*, 2001; Iso *et al.*, 2001; Ohto *et al.*, 2002); most likely the virus enters the child's body shortly after birth.

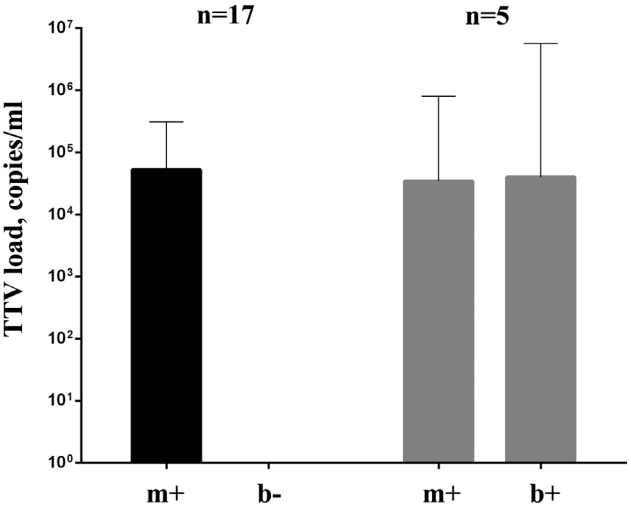

Fig. 3

Comparison of TTV loads in mother (+) baby (-) and mother (+) baby (+) mother-child pairs
In mother-child pairs (n = 22) with TTV-positive mothers (m+), the infants were TTV-negative (b-, n = 17) and TTV-positive (b+, n = 5).

Per contra, a number of studies indicate the presence of TTV DNA in 1–66% of cord blood samples (Saback *et al.*, 1999; Gerner *et al.*, 2000; Goto *et al.*, 2000; Kazi *et al.*, 2000; Morrica *et al.*, 2000; Matsubara *et al.*, 2001; Zhong *et al.*, 2001; Xin *et al.*, 2004; Mutlu *et al.*, 2007). Such dif-

ferences can be explained either by low PCR sensitivity (for a TTV-negative cord blood studies) or by contamination with PCR products (since TTV-positive cord blood was usually detected by contamination-friendly nested PCR) or by mother's blood contamination at the sample collection step.

The viral genome sequences comparison is an additional argument in favor of postpartum acquisition of TTV in neonates. In about half of the mother-child pairs, infant TTV isolates differ from maternal ones (Zhong *et al.,* 2001; Bagaglio *et al.,* 2002; Lin *et al.,* 2002; Ohto *et al.,* 2002). Our study demonstrates no statistically significant difference in TTV viral load of mothers whose children were TTV-positive or TTV-negative. This is consistent with a study (Mutlu *et al.,* 2007) demonstrating no difference in TTV load between mothers who passed and did not transmit TTV to their children. Also, there was no statistically significant difference in TTV viral load between term and preterm infants (data not shown).

Breast milk is often TTV-positive (23–67%) (Iso *et al.,* 2001; Matsubara *et al.,* 2001) and therefore may also be one of the transmission routes for TTV. But our data do not show a statistically significant difference in TTV viral load based on breastfeeding (data not shown).

Our data showed no difference in TTV viral load depending on delivery mode (data not shown). Interestingly, the study of McCann *et al.* (2018) found a difference between the amount of TTV in a feces samples and the mode of delivery, while they also did not observe significant differences in the feeding type. They also suggest that TTV is transmitted not only through the birth canal and breastfeeding, but also from the environment and through tactile contact with parents and others.

Current results indicate that the rates of transmission and/or proliferation of TTV are independent of maternal viral load, term, delivery type and breastfeeding. The observed diversity in TTV dynamics may reflect the diversity of emerging immune response patterns in young children.

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