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A Higher Incidence of Isolated Biliary Atresia in Rural Areas: Results From an Epidemiological Study in The Netherlands

*Mark Nomden, *Daan B.E. van Wessel, †Solomon Ioannou, ‡Henkjan J. Verkade, §Ruben H. de Kleine, †Behrooz Z. Alizadeh, *Janneke L.M. Bruggink, and *Jan B.F. Hulscher

ABSTRACT

Objectives: Environmental factors may be involved in the pathogenesis of biliary atresia (BA). This epidemiological study aimed to analyze the relationships between the incidence of BA, the incidence of confirmed viral or bacterial infections and population density, and geographical and temporal clustering of BA in the Netherlands.

Study Design: Correlations between the monthly incidence of BA and the number of confirmed infections were assessed. BA incidence per province was calculated and compared to the province with highest population density. Birthplaces were classified as rural or urban. Temporal clustering of month of birth and month of conception were analyzed. We performed analyses for isolated BA (IBA) and syndromic BA (SBA) separately. Chi^2, logistic regression, and Walter and Elwood test were used.

Results: A total of 262 IBA and 49 SBA patients, born between 1987 and 2018, were included. IBA incidence correlated to the number of confirmed infections of, for example, *Chlamydia trachomatis* ($R = 0.14$; $P = 0.02$) and adenovirus ($R = 0.22$; $P = 0.005$). We observed a higher incidence of IBA (0.75/10,000; odds ratio [OR] = 1.86; $P = 0.04$) and SBA (0.27/10,000; OR = 6.91; $P = 0.001$) in Groningen and a higher incidence of SBA in Gelderland (0.13/10,000; OR = 3.35; $P = 0.03$). IBA incidence was 68% higher in rural (0.67/10,000) versus urban areas (0.40/10,000) ($P = 0.02$). The estimated month of conception of patients with SBA clustered in November (85% increase compared to average SBA incidence [0.09/10,000; $P = 0.04$]).

Conclusions: IBA incidence correlated weakly with national confirmed infections. IBA and SBA incidence varied geographically in the Netherlands. IBA incidence was higher in rural than in urban areas, which may be explained decreased exposure to pathogens. Our results provide support for a role of environmental factors in the pathogenesis of IBA.

Key Words: cholangiopathy, clustering, infections, seasonality, urbanicity (*JPGN* 2021;72: 202–209)

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From the *Section of Paediatric Surgery, Department of Surgery, University Medical Centre Groningen, the †Department of Epidemiology, University Medical Centre Groningen, the ‡Section of Paediatric Gastroenterology and Hepatology, Department of Paediatrics, Beatrix Children's Hospital, University Medical Centre Groningen, and the §Section of Hepato-pancreatic-biliary Surgery and Liver Transplantation, Department of Surgery, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands.

Address correspondence and reprint requests to Mark Nomden, BSc, Department of Paediatric Surgery University Medical Centre Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands (e-mail: m.nomden@umcg.nl).

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What Is Known

- Environmental factors, such as viral infections, have been associated in the pathogenesis of isolated biliary atresia.
- Studies investigating geographical and temporal clustering have yielded varying results.

What Is New

- The incidence of isolated biliary atresia correlated with pathogens, for example, adenovirus and *Chlamydia trachomatis.*
- Geographical clustering of isolated and syndromic biliary atresia was observed in 1 and 2 provinces in the Netherlands, respectively.
- The incidence of isolated biliary atresia was significantly higher in rural areas compared to urban areas.
- Temporal clustering of isolated biliary atresia was not observed.

Biliary atresia (BA) is a rare cholangiopathy of infancy, characterized by cholestasis and pale stools in the first weeks of life. Its incidence varies from approximately 1:8000 newborns in Asian countries to 1:20,000 in European countries (1,2). BA exists in an isolated BA (IBA) and syndromic BA (SBA) form, accounting for approximately 80% and 20% of cases, respectively. IBA is characterized by obliteration of the intra- and extrahepatic bile ducts. SBA is, in addition to the obliteration of the intra- and extrahepatic bile ducts, characterized by congenital malformations such as polysplenia, situs inversus and malrotation (1,3,4). The cause of either IBA or SBA is unknown. Mutations in specific genes have been associated with both IBA and SBA, such as the intercellular adhesion molecule-1 in IBA and polycystic kidney disease 1 like 1 gene, a gene associated with embryonic laterality, in SBA (5,6). They play a role in inflammation and determination of the left-right axis in embryonic development, respectively. A large cohort is still required to confirm the involvement of these genes in the pathophysiology of BA. A popular hypothesis for IBA describes an infectious insult as an initiator of bile duct injury (1,5,7). Subsequently, an exaggerated immune response results in bile duct obliteration (5). *Rotavirus* (RV) (8,9), reovirus (10), and cytomegalovirus (11,12) have been proposed as the inducing pathogens. Moreover, rhesus RV has successfully been used to generate experimental BA in animal models, supporting the hypothesis of an infectious insult as the initiator of the exaggerated immune response (13,14). SBA, on the contrary, is thought to be caused by a developmental error, rather than due to an environmental insult (1).

This is supported by the observed laterality defects in SBA, such as situs inversus or intestinal malrotation (3).

If the origin of IBA would indeed be (partially) attributable to an environmental or infectious insult during pregnancy or in the perinatal period, one could expect differences in geographical distribution and in the distribution over the months in a year of the incidence of IBA, while this is not expected in SBA, due to its developmental origin. Studies conducted in cohorts in which patients with IBA and SBA had been combined provide conflicting results with regard to geographical and seasonal clustering (15–21). Moreover, supporting data regarding environmental factors, such as bacterial and viral infections in the environment or population density, are lacking in the majority of these studies. The current study aimed first to analyze the relationships between the incidence of IBA and SBA and national counts of confirmed bacterial and viral infections in the Netherlands. Secondly, we aimed to assess whether geographical and/or temporal clustering of IBA and SBA exists. These data could strengthen or weaken the hypothesized involvement of an environmental factor in the pathogenesis of IBA and SBA.

PATIENTS AND METHODS

Study Design and Patient Inclusion

This nationwide, ecological cohort study was performed in accordance with the guidelines of the Medical Ethical Committee of the University Medical Centre Groningen (2017/056). For this study, we used the Netherlands Study Group on Biliary Atresia Registry (NeSBAR) database, which contains demographic data, perioperative data, and long-term follow-up data of all patients with confirmed BA in the Netherlands since 1987. We selected all patients with BA born between January 1987 and December 2018. We classified patients as having SBA when one of the associated congenital malformations was present, for example, polysplenia, situs inversus, and intestinal malrotation (1,3). Patients were excluded if their place of birth was outside the Netherlands (n = 7) or in case of missing data (n = 7). We performed analyses for IBA and SBA separately. We calculated the incidence of IBA and SBA by dividing the number of BA cases within the respective period of time by the number of live births in the same time span, expressed as cases per 10,000 live births.

Pregnancy

Based on the theory that IBA originates in the gestational period (22), we assessed the relationship between the month of conception, or the month of bile duct formation on the one hand, and number of confirmed viral or bacterial infections on the other hand. We estimated the month of conception per BA patient by subtracting their gestational age from their date of birth. We calculated the estimated number of conceptions of BA children per month per total number of live births. This was termed the ''incidence of conception of BA patients.'' Because the intra- and extrahepatic bile ducts start to develop between 4 and 8 weeks of life in the human embryo (3), we estimated the month during which bile duct formation took place. This was calculated by adding 6 weeks to the date of estimated conception (which was calculated as stated previously). We made an estimation of the number of active pregnancies in which bile duct formation was taking place. An incidence of the formation of bile ducts in BA children was calculated by dividing the number of BA pregnancies by the estimated total number of pregnancies during each month. We expressed the incidence as number of BA patients per 10,000 pregnancies. This incidence was used to assess if there was a correlation between the number of confirmed infections and the formation of bile ducts in patients with BA.

Confirmed Viral and Bacterial Infections in Relation to the Incidence of Biliary Atresia and Active Biliary Atresia Pregnancies

We acquired the weekly number of new confirmed viral and bacterial infections in the Netherlands from the National Institute of Public Health and the Environment (RIVM). Twenty-one laboratories in the Netherlands report the number of new confirmed viral and bacterial infections per week to the RIVM. These data give reliable information about the national abundance of viruses and bacteria and can be used to assess national trends of infections in the Netherlands (23). The RIVM can use these data to monitor the abundance of infections in the environment to be able to take protective measures for public health when needed. We now applied these data for the first time to address a potential correlation with the incidence of a rare disease, in this case the incidence of IBA and SBA. We estimated the number of reproductive women per year based on the number of women at risk of acquiring periconceptual or perinatal infections of 20 to 50 years of age from the central bureau of statistics (CBS). We calculated the number of bacterial and viral infections per month per reproductive women by dividing the number of infections per separate pathogen in a particular month by the number of reproductive women per year. We analyzed the relationship between the incidences of IBA and SBA to the calculated infections per 10,000 reproductive women.

Geographical Clustering

We obtained the number of live births per province per year from the CBS. The incidence of IBA and SBA per province per year was calculated by dividing the number of BA cases per year by the number of live births per year, per province (expressed as the number of BA cases per 10,000 live births per year, per province). Because a high population density is associated with an increased risk of acquiring an infection (24), we chose the province with the highest population density (ie, South Holland) as a reference. To associate the incidence of BA with population density, we retrieved the degree of urbanicity per municipality from 1997 until 2019 from the CBS. The degree of urbanicity is based on the average area address density per municipality. The degree of urbanicity is categorized into 5 groups by the CBS: ''no urbanicity'' (<500 addresses/km^2), ''low urbanicity'' (500–1000 addresses/km^2), ''moderate urbanicity'' (1000–1500 addresses/km^2), ''high urbanicity'' (1500–2500 addresses/km^2), and ''very high urbanicity'' (>2500 addresses/km^2) (25). We allocated patients in 1 of these 5 urbanicity groups, according to the address density of the residential zip code at time of birth. We calculated BA incidences per urbanicity group. We included patients born between 1997 and 2018 in this analysis, since data regarding the number of live births per municipality were only available from 1997 onwards. To compare the incidence IBA or SBA between ''extreme'' rural and ''extreme'' urban areas, we compared the group of ''no urbanicity'' with the group of ''high urbanicity'' and ''very high urbanicity'' combined, respectively. We disregarded the 2 groups of low and moderate urbanicity, because these groups could not be categorized as truly rural or truly urban as per definition by the CBS (26). Moreover, by excluding these 2 groups we correct for changes in urbanicity over a long period of time. Rural areas became more rural over time while urban areas became more urban over the time of the study (26).

Temporal Clustering

We calculated the accumulated monthly incidence of IBA and SBA by dividing the total number of IBA and SBA cases per respective month within the study period by the total number of live births per respective month in the study period. We expressed the accumulated monthly incidence of each subtype as cases per 10,000 live births per month. Patients included in this analysis were born between January 1995 and December 2018, because data regarding live births per month were available from 1995 onwards.

Statistics

All variables are expressed as number of BA cases per 10,000 live births (incidence/10,000). To assess the correlation between BA incidence and number of confirmed infections per 10,000 reproductive women, we used Spearman or Pearson correlations as appropriate. We used logistic regression analysis to assess spatial clustering per province and this was expressed as odds ratios (ORs). The Chi^2 test was used to compare clustering between urbanicity groups and between ''truly rural'' and ''truly urban'' areas. We used Walter and Elwood (27) test of seasonality to assess temporal clustering. In case of missing data, patients were excluded from the respective analysis. The number of patients within each analysis was provided. We considered a P value <0.05 statistically significant. In the correlation analysis, we chose not to correct the statistical significance level for multiple testing due to the explorative nature of this study and thereby prevent type II statistical errors. All analyses were performed using IBM SPSS Statistics 23.0 (Armonk, NY) and R statistical package 3.5.3. We constructed figures using Prism 7.02 (GraphPad Software, La Jolla, CA).

RESULTS

Between January 1987 and December 2018, 311 patients (139 boys, 45%) with BA were born. Out of 311 patients 262 (84%) had IBA (118 boys, 45%) and 49/311 (16%) SBA (21 boys, 43%). There were 6.017.136 live births in the Netherlands during the study period. Therefore, the incidence of BA was 0.52 of 10,000 live births (1/19,231).

The Relationship Between Biliary Atresia Incidence and Confirmed Viral or Bacterial Infections

The monthly incidence of IBA correlated weakly, yet significantly to pathogens such as adenovirus, *Chlamydia trachomatis*, *Coxiella*, hepatitis E virus (HEV), influenza A and B virus, rhinovirus, *Rickettsia* virus, and *Sapovirus* (R coefficient between 0.12 and 0.24; P values between 0.004 and 0.047, Supplementary Table 1, *http://links.lww.comd/MPG/B950*). We observed weak, yet statistically significant correlations between the month of conception of IBA children and, *Chlamydia*, dengue virus, HEV, *Norovirus*, and untyped parainfluenza virus (R coefficient between -0.18 and 0.17; P values between 0.02 and 0.04, Table Supplementary Table 1, *http://links.lww.com/MPG/B950*). The incidence of the conception of patients with SBA correlated negatively with *Sapovirus* ($R=-0.34$, $P=0.002$). The month of approximate bile duct formation in patients with IBA correlated weakly, yet significantly with, among others, adenovirus; *C pneumoniae*; HEV *Norovirus*; and parainfluenza type 2, type 3, and untyped parainfluenza virus (R coefficient between -0.18 and 0.16; P values between 0.003 and 0.05, Supplementary Table 1, *http://links.lww.com/MPG/B950*). The month of approximate bile duct formation in patients with SBA correlated significantly with untyped *Chlamydia* ($R=0.121$, $P=0.04$) and *Sapovirus* ($R=-0.29$; $P=0.009$). We did not observe significant correlations between the incidences of IBA or SBA and RV.

The Relationship Between Biliary Atresia Incidence, Geographical Location, and Population Density

The incidence of IBA and SBA was compared between the 12 provinces of the Netherlands. Of these, the province of South Holland has the highest population density and was therefore used as reference. A map of the Dutch provinces and incidence of BA is shown in supplementary Figure 1, *http://links.lww.com/MPG/B947*. We observed a higher incidence of IBA in the province of Groningen (0.75/10,000 live births, OR: 1.86, $P=0.04$, Table 1). The incidence of SBA in the provinces of Groningen (0.27/10,000 live births, OR: 6.91, $P=0.002$) and Gelderland (0.13/10,000 live births, OR: 3.35, $P=0.03$) was significantly higher compared to the province of South Holland.

Supplementary Figure 2, *http://links.lww.com/MPG/B948* depicts the incidence of BA per urbanicity group (ie, 5 groups based on the average address density per municipality). In total, we included 202 patient with BA in this analysis (IBA n = 164 and SBA n = 38) because data from the CBS regarding urbanicity were available from 1997 onwards. We observed an overall significant difference in the incidence of IBA of the 5 urbanicity groups ($P=0.02$, Supplementary Fig. 2, *http://links.lww.com/MPG/B948*). The highest incidence of IBA was observed in the group of ''no urbanicity'' (0.67/10,000 live births), whereas the lowest incidence of IBA was observed in the group of ''low urbanicity'' (0.27/10,000 live births). We did not observe a significant difference in the incidence of SBA within the same groups ($P=0.08$). The highest incidence of SBA was found in the group of ''no urbanicity'' (0.13/10,000 live births), whereas the lowest incidence was observed in the group of ''high urbanicity'' (0.03/10,000 live births).

To scrutinize where the exact differences originated from, we compared incidences of IBA and SBA between the extremes of urbanicity: that is, the group of ''no urbanicity'' versus the ''high'' and ''very high urbanicity'' groups (definitions detailed in Methods).The incidence of IBA in truly rural (0.67/10,000 live births) areas was significantly higher compared to truly urban areas (0.40/10,000 live births) (OR = 1.65, $P=0.02$, Fig. 1). The incidence of SBA was higher in truly rural areas (0.13/10,000 live births) than in urban areas (0.06/10,000 live births) (OR = 2.07; $P=0.13$, Fig. 1). An overview of the geographical location of BA cases is shown in Figure 2.

Temporal Clustering in Isolated Biliary Atresia Was Not Observed, Yet the Month in Which Patients With Syndromic Biliary Atresia Were Conceived Was Highest in November

To assess temporal clustering of IBA and SBA, we used Walter and Elwood test of seasonality which is used to test the seasonality of a binary outcome with a variable population at risk (28). There was no significant difference in incidence per month for IBA (supplementary Fig. 3, *http://links.lww.com/MPG/B949*). Patients with SBA were most often conceived in November (0.16/10,000, Fig. 3). The incidence was 85% higher in November as compared to the average incidence of SBA (0.09/10,000 live births). We observed significant seasonality as measured by the

TABLE 1. Incidence of isolated biliary atresia (n = 252) and syndromic biliary atresia (n = 49) per province in the Netherlands

Province	Incidence IBA Expressed per 10,000 live births	OR IBA (95% CI) relative to South Holland	Incidence SBA Expressed per 10,000 live births	OR SBA (95% CI) relative to South Holland
South Holland	0.40 (52/1,290,319)	1.00	0.04 (5/1,290,319)	1.00
Friesland	0.49 (11/222,810)	1.23 (0.64–2.35) $P = 0.54$	0.09 (2/222,810)	2.32 (0.45–11.94) $P = 0.32$
Drenthe	0.38 (6/156,295)	0.95 (0.41–2.22) $P = 0.91$	0.06 (1/156,295)	1.65 (0.19–14.13) $P = 0.65$
Overijssel	0.41 (17/413,563)	1.02 (0.59–1.76) $P = 0.94$	0.10 (4/413,563)	2.50 (0.67–9.30) $P = 0.17$
Flevoland	0.27 (4/146,103)	0.68 (0.25–1.89) $P = 0.46$	0.07 (1/146,103)	1.77 (0.21–15.12) $P = 0.60$
Gelderland	0.45 (31/693,211)	1.11 (0.71–1.73) $P = 0.65$	0.13 (9/693,211)	3.35 (1.12–10.00) $P = 0.03$
Utrecht	0.57 (26/459,888)	1.46 (0.92–2.32) $P = 0.11$	0.02 (1/459,888)	0.56 (0.07–4.80) $P = 0.60$
North Holland	0.41 (39/961,542)	0.98 (0.64–1.49) $P = 0.93$	0.09 (9/961,542)	2.15 (0.70–6.56) $P = 0.18$
Groningen	0.75 (14/186,689)	1.86 (1.03–3.36) $P = 0.04$	0.27 (5/186,689)	6.91 (2.00–23.87) $P = 0.002$
Zeeland	0.64 (8/125,256)	1.59 (0.75–3.34) $P = 0.23$	0 (0/125,256)	0.00 $P = 0.94$
North Brabant	0.42 (35/834,182)	1.03 (0.68–1.60) $P = 0.85$	0.11 (9/834,182)	2.78 (0.93–8.31) $P = 0.07$
Limburg	0.26 (9/339,979)	0.68 (0.32–1.33) $P = 0.24$	0.09 (3/339,979)	3.04 (0.82–11.31) $P = 0.10$

Incidence is expressed as cases per 10,000 live births. ORs are calculated relative to the province of South Holland.
CI = confidence interval; IBA = isolated biliary atresia; OR = odds ratio; SBA = syndromic biliary atresia.

Walter and Elwood test ($P = 0.04$), although with a moderate goodness of fit test ($P = 0.43$), indicating that seasonality is unlikely.

DISCUSSION

The aim of this study was to assess the possible relationships between the incidence of IBA and SBA and number of confirmed viral and bacterial infections in the Netherlands. In addition, we aimed to assess whether the incidence of IBA and SBA were geographically and temporal clustered in the Netherlands, to provide insights in the possible role of environmental factors in the pathogenesis of IBA. The present study indicated that the incidence of IBA and SBA was only weakly correlated with, for example, adenovirus and *C trachomatis*. We observed an increased incidence of IBA and SBA in 2 provinces of the Netherlands. Moreover, the incidence of IBA was significantly higher in rural areas compared to urban areas.

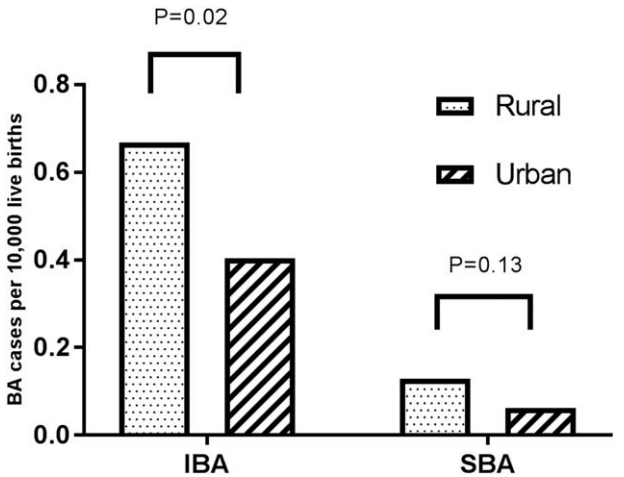

FIGURE 1. The incidence of isolated BA (IBA, n = 115) and syndromic BA (SBA, n = 19) per 10,000 live births in rural (dotted bars) and urban areas (dashed bars). Definitions: rural: group of no urbanicity (<500 addresses/km^2; urban: group of high urbancity (1500–2500 addresses/km^2) and very high urbancity (>2500 addresses/km^2) combined. IBA = isolated biliary atresia; SBA = syndromic biliary atresia.

Incidence of Biliary Atresia and Confirmed Number of Confirmed Viral or Bacterial Infections

Several studies have described an association between BA and RV (8,9,29), but our present data did not confirm this association. An explanation for this finding may be that different RV strains induce experimental BA with varying efficacy, which suggests that strain type may be important (30,31) in its pathogenicity. This is illustrated by Danial et al (32), who observed declining rates of RV-A infections after vaccination against RV-A, whereas the prevalence of BA increased. In the absence of RV-A, other RV strains may become relatively more abundant and potentially cause a rise in BA incidence. In Taiwan a vaccine was used that protected against RV-A and partly against RV type C (33). Lin et al (33) observed that the incidence of BA decreased with increasing vaccination rates, although their results were borderline significant.

In this study, we observed significant associations between the incidence of IBA and confirmed number of infections of adenovirus, HEV, *C trachomatis*, and dengue virus, among

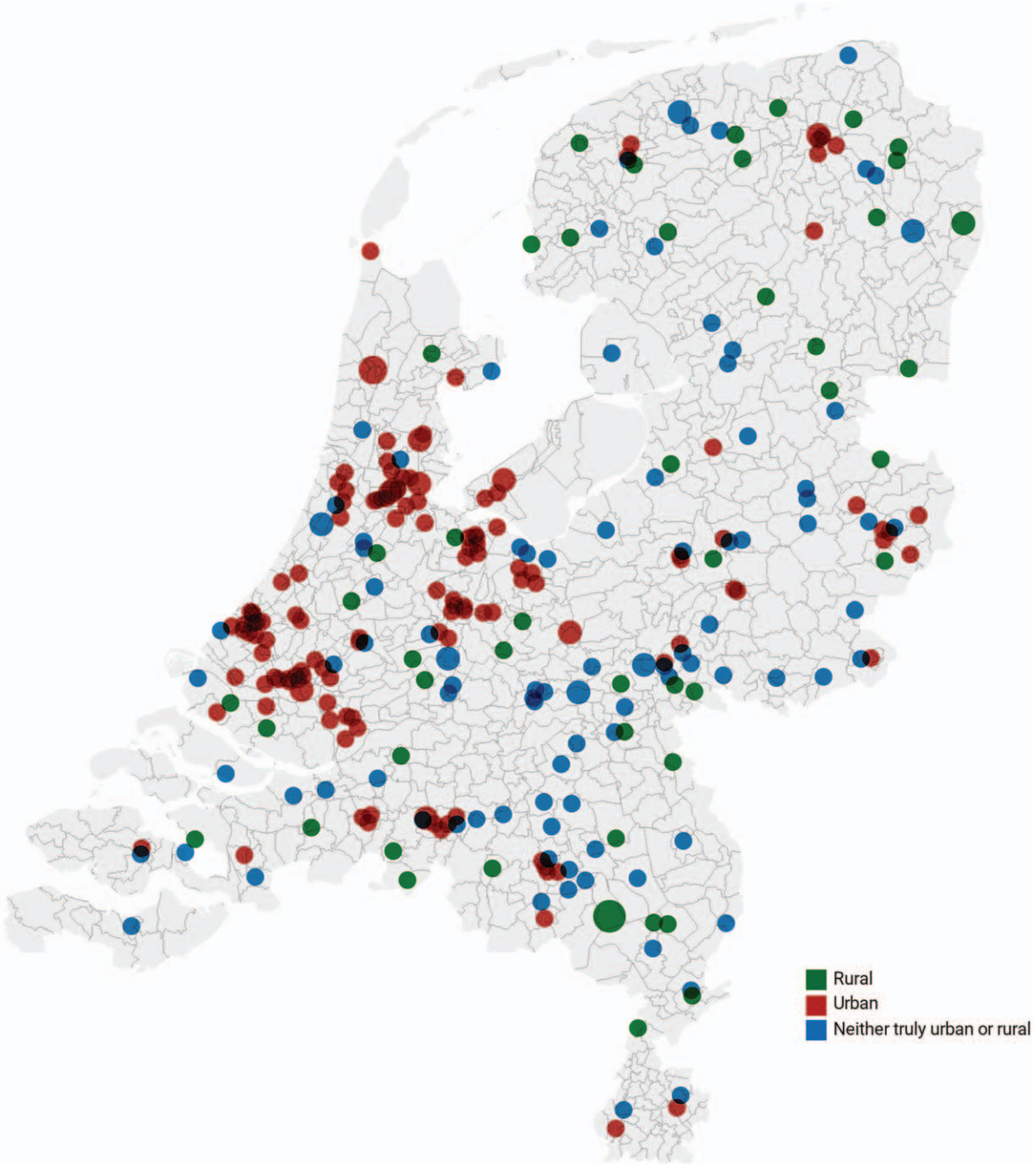

FIGURE 2. The geographical location of overall BA cases in the Netherlands. Dots represent the location of cases. A bigger dot size represents multiple cases of BA within the same zip code (min: 1 case, max: 4 cases). Different colors represent rural (green) and urban (red) areas. Areas neither truly rural nor truly urban are represented in blue. The map of the Netherlands is divided into 3 digit zip code areas. No distinction between isolated BA and syndromic BA was made.

others. DNA and RNA of these hepatotropic viruses has been demonstrated in liver biopsies of patients with BA taken during Kasai portoenterostomy (11,34), yet this may have been acquired after BA developed. Further study into these pathogens might therefore be warranted. *C trachomatis* has not been routinely associated with BA. In a study by Ponsioen et al (35), higher serum levels of *Chlamydia*-specific antibodies were observed in patients with BA compared to children with primary sclerosing cholangitis or other cholestatic diseases. *C trachomatis* may inflict autoimmune-mediated injury by molecular mimicry of the human heat shock protein-60, as demonstrated in pelvic inflammatory disease (36). A correlation between the incidence of IBA in the month of conception and dengue virus was observed in our study. An epidemiological study in Taiwan reported an

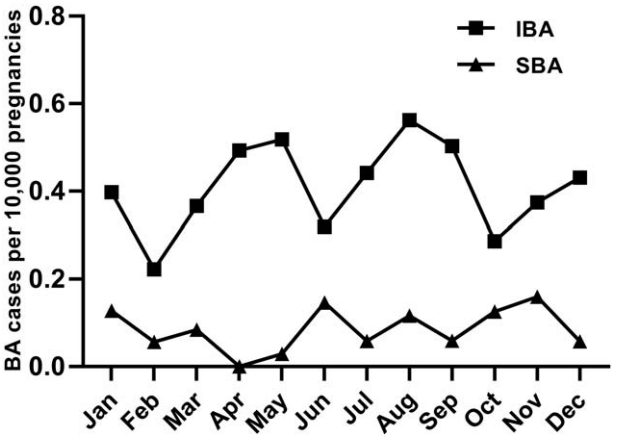

FIGURE 3. The incidence of isolated BA (IBA, n = 176, squares) and syndromic BA (SBA, n = 37, triangles) per month of conception per 10,000 pregnancies. IBA = isolated biliary atresia; SBA = syndromic biliary atresia

increase in the incidence of BA during a dengue virus epidemic (37). Although dengue virus is prevalent in tropical regions and not often seen in colder regions, such as Northern Europe, the relation between dengue virus and BA may only be of interest from a pathologic point of view.

The innate immune responses elicited by these pathogens have been investigated by several studies, which showed that toll-like-receptors (TLRs), such as TLR4 and TLR2 were activated by adenovirus, *C trachomatis*, dengue virus, and HEV (38–41). Moreover, IBA is characterized by a T-helper (Th) 1 cellular response (5). Via activation of TLR4 and TLR2, adenovirus, *C trachomatis*, dengue virus, and HEV are also able to elicit a Th1-dominant inflammation and may thus play a role in the pathogenesis of IBA.

Our study also showed that there were negative correlations between the incidence of BA and the incidence of certain bacterial or viral infection, such as *Sapovirus*. This further supports that there is an association with an environmental factor and the incidence of BA. A negative correlation would indicate a protective effect of an infection with that specific pathogen or can be due to an unidentified common factor that affects both the incidence of that specific pathogen and the incidence of BA.

Geographical Clustering

Our data indicate that the incidence of IBA is higher in rural areas. In agreement with our study, Strickland and Shannon (18) and Fischler et al (42) observed a higher incidence of BA in rural compared to urban areas. On the contrary, Chardot et al (15) failed to observe a relationship between the BA incidence and population density. They however, calculated population density per province instead of per municipality or zip code. We hypothesized that, if IBA is indeed caused by an environmental factor such as an infectious insult, we would observe higher incidences of IBA in urban areas, since one's risk of acquiring an infection is generally higher in densely populated areas (24). Our findings may be explained by the ''hygiene theory'' or ''microflora hypothesis'' (43,44). These theories state that high sanitary standards limit exposure to microorganisms, which is required to establish a rich and balanced gut microbiota (ie, the collection of bacteria, viruses, fungi, and archaea in the gut). The exposure to microorganisms may be lower in rural areas due to low population density. Whether or not the gut microbiota indeed play a role in the pathogenesis of BA needs to be established in future studies, yet 1 study already established a difference in the microbiome composition of healthy and BA infants (45). Moreover, in other (adult) cholestatic liver diseases it seems that a disrupted microbiome may initiate or aggravate liver injury (46,47).In these diseases, for example in primary sclerosing cholangitis, it has been shown that bacterial translocation leads to TLR4 activation and a subsequent Th1 dominant biliary inflammation. This supports the hypothesis that a Th1-dominant response can be elicited in BA by activation of a TLR.

Temporal Clustering

Temporal clustering of IBA was not observed. This is in accordance with most European and Northern American studies (15,16,19,21,42). In Korea and Texas state (USA), the BA incidence was, however, higher in the summer months (18,48). On the contrary, Yoon et al (20) and Caton et al (17) observed a higher incidence of BA in the winter months, which is consistent with a viral etiology of RV, for example. These studies further support that an environmental factor may be at play in the etiology of IBA (17,18,20,48). The fact that we were unable to replicate these results may be due to factors such as a difference in ethnicity, which can lead to different susceptibility or reaction to viruses for example (49,50). Moreover, it may be that more than 1 pathogen is involved in the pathogenesis of IBA, which affects temporal distribution of IBA cases.

Surprisingly, our study showed that patients with SBA were most often conceived in November: an almost 2-fold increase of incidence occurred as compared to the average incidence of SBA. It is thought that SBA is caused by a developmental error during the fetal period, rather than due involvement of an infectious or environmental factor (1). *Chlamydia* was, however, associated with SBA in the month of approximate bile duct formation. Existing literature (2,4) describes complications during the pregnancy of SBA children that could play a role in the pathogenesis, yet this includes endocrine (eg, maternal diabetes) rather than infectious complications. It may be that clustering of SBA is coincidental, related to the still relatively small SBA cohort. This is further supported a moderate goodness of fit test, indicating that the results of the W&E test of seasonality should be interpreted with caution.

We are aware of the limitations of our study. Unfortunately, number of confirmed infections regarding viruses that have previously been associated with BA (ie, cytomegalovirus and reovirus) were not available from the national institute for Public Health and the Environment (RIVM). We lacked information regarding, for example, RV and adenovirus subtypes. Also, the reported pathogen counts were national counts, rather than counts per province or per municipality. Our correlation coefficients between the incidence of BA and the number of confirmed viral and bacterial infections were relatively low, which might have been caused by the rarity of BA. During the study period, we did not observe BA in the majority of individual months, which led us to include a significant proportion of ''0'' values in our calculations. The observed correlation coefficients may therefore be underestimations. According to the CBS, the peripheral (ie, rural) parts of municipalities have a shortage of young adults and births, whereas the opposite is true for urban areas (26). This results in an overestimation of the number of births in the rural parts and an underestimation of the number of births in the urban parts of the respective municipality. Therefore, the incidence of BA is likely

under- and overestimated for rural and urban areas, respectively. Hence, the differences between BA incidence in rural and urban areas may actually have been underestimated. Ethnicity is implicated in the incidence of BA (1) and analyzing ethnicity in our patient cohort would have been a useful addition in interpreting this data. Unfortunately, ethnicity data had not been collected in the NeSBAR database. Despite these limitations, our study was the first to study the association between the incidence of BA and national counts of confirmed infections. Temporal clustering of BA cases was analyzed at multiple time points during pregnancy of BA children.

In conclusion, our study found an association between the incidence of BA and national number of confirmed viral or bacterial infections, such as *C trachomatis* and dengue virus. The incidence of IBA was higher in rural areas as opposed to urban areas. Temporal clustering of IBA was not observed, yet the month of conception of patients with SBA clustered in time. Our findings provide support for the hypothesis that 1 (or more) environmental factor(s) may be involved in the pathogenesis of IBA.

REFERENCES

1. Hartley JL, Davenport M, Kelly DA. Biliary atresia. *Lancet (London, England)* 2009;374:1704–13.
2. Lakshminarayanan B, Davenport M. Biliary atresia: a comprehensive review. *J Autoimmun* 2016;73:1–9.
3. Davenport M. Biliary atresia: from Australia to the zebrafish. *J Pediatr Surg* 2016;51:200–5.
4. Davenport M. Biliary atresia: clinical aspects. *Semin Pediatr Surg* 2012;21:175–84.
5. Mack CL, Feldman AG, Sokol RJ. Clues to the etiology of bile duct injury in biliary atresia. *Semin Liver Dis* 2012;32:307–16.
6. Berauer J-P, Mezina AI, Okou DT, et al. Identification of polycystic kidney disease 1 like 1 gene variants in children with biliary atresia splenic malformation syndrome. *Hepatology* 2019;70:899–910.
7. Wehrman A, Waisbourd-Zinman O, Wells RG. Recent advances in understanding biliary atresia. *F1000Res* 2019;8:F1000.
8. Riepenhoff-Talty M, Gouvea V, Evans MJ, et al. Detection of group C rotavirus in infants with extrahepatic biliary atresia. *J Infect Dis* 1996;174:8–15.
9. Clemente MG, Patton JT, Yolken R, et al. Prevalence of groups A and C rotavirus antibodies in infants with biliary atresia and cholestatic controls. *J Pediatr* 2015;166:79–84.
10. Tyler KL, Sokol RJ, Oberhaus SM, et al. Detection of reovirus RNA in hepatobiliary tissues from patients with extrahepatic biliary atresia and choledochal cysts. *Hepatology* 1998;27:1475–82.
11. Xu Y, Yu J, Zhang R, et al. The perinatal infection of cytomegalovirus is an important etiology for biliary atresia in China. *Clin Pediatr (Phila)* 2012;51:109–13.
12. De Tommaso AMA, Andrade PD, Costa SCB, et al. High frequency of human cytomegalovirus DNA in the liver of infants with extrahepatic neonatal cholestasis. *BMC Infect Dis* 2005;5:108.
13. Riepenhoff-talty M, Schaekel K, Clark HF, et al. Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. *Pediatr Res* 1993;33:394–9.
14. Mohanty SK, Donnelly B, Temple H, et al. A rotavirus-induced mouse model to study biliary atresia and neonatal cholestasis. *Methods Mol Biol* 2019;1981:259–71.
15. Chardot C, Carton M, Spire-Bendelac N, et al. Epidemiology of biliary atresia in France: a national study 1986–96. *J Hepatol* 1999; 31:1006–13.
16. Livesey E, Cortina Borja M, Sharif K, et al. Epidemiology of biliary atresia in England and Wales (1999–2006). *Arch Dis Child Fetal Neonatal Ed* 2009;94:F451–5.
17. Caton AR, Druschel CM, McNutt LA. The epidemiology of extrahepatic biliary atresia in New York State, 1983–98. *Paediatr Perinat Epidemiol* 2004;18:97–105.
18. Strickland AD, Shannon K. Studies in the etiology of extrahepatic biliary atresia: time-space clustering. *J Pediatr* 1982;100:749–53.
19. Houwen RH, Kerremans II, Van Steensel-Moll HA, et al. Time-space distribution of extrahepatic biliary atresia in The Netherlands and West Germany. *Z Kinderchir* 1988;43:68–71.
20. Yoon PW, Bresee JS, Olney RS, et al. Epidemiology of biliary atresia: a population-based study. *Pediatrics* 1997;99:376–82.
21. Wada H, Muraji T, Yokoi A, et al. Insignificant seasonal and geographical variation in incidence of biliary atresia in Japan: a regional survey of over 20 years. *J Pediatr Surg* 2007;42:2090–2.
22. Mysore KR, Shneider BL, Harpavat S. Biliary atresia as a disease starting in utero: implications for treatment, diagnosis, and pathogenesis. *J Pediatr Gastroenterol Nutr* 2019;69:396–403.
23. Van Dijk M, Mooij S, Duijster J et al. Evaluatie van de virologische weekstaten. Infectieziekten bulletin, RIVM. *https://magazines.rivm.nl/2019/03/infectieziekten-bulletin/evaluatie-van-de-virologische-weekstaten*. Published 2019. Accessed October 2019.
24. Jones KE, Patel NG, Levy MA, et al. Global trends in emerging infectious diseases. *Nature* 2008;451:990–3.
25. Statistics CB of Urbanicity. *https://www.cbs.nl/nl-nl/artikelen/nieuws/2019/44/meeste-afval-per-inwoner-in-minst-stedelijke-gemeenten/stedelijkheid*. Accessed October 11, 2019.
26. Steenbekkers A, Vermeij L, van Houwelingen P. Dorpsleven tussen stad en land. Final publication social status of rural areas. *Den Haag: Sociaal en Cultureel Planbureau (SCP)*. Accessed via https://www.binnenlandsbestuur.nl/Uploads/2017/3/Dorpsleven-tussen-stad-en-land-WEB.pdf. Accessed October 2019.
27. Walter SD, Elwood JM. A test for seasonality of events with a variable population at risk. *Br J Prev Soc Med* 1975;29:18–21.
28. Nyári TA, Virag K, McNally RJQ. Analysis of double-peak seasonality in the aetiology of perinatal mortality and childhood acute lymphoblastic leukaemia using the Walter-Elwood method. *Appl Ecol Environ Res* 2019;17:3941–8.
29. Keyzer-Dekker CM, Lind RC, Kuebler JF, et al. Liver fibrosis during the development of biliary atresia: proof of principle in the murine model. *J Pediatr Surg* 2015;50:1304–9.
30. Allen SR, Jafri M, Donnelly B, et al. Effect of rotavirus strain on the murine model of biliary atresia. *J Virol* 2007;81:1671–9.
31. Hertel PM, Estes MK. Rotavirus and biliary atresia: can causation be proven? *Curr Opin Gastroenterol* 2012;28:10–7.
32. Danial E, Fleck-Derderian S, Rosenthal P. Has rotavirus vaccination decreased the prevalence of biliary atresia? *J Clin Gastroenterol* 2019;53:e348–51.
33. Lin Y-C, Chang M-H, Liao S-F, et al. Decreasing rate of biliary atresia in Taiwan: a survey. *Pediatrics* 2011;128:e530–6.
34. Rauschenfels S, Krassmann M, Al-Masri AN, et al. Incidence of hepatotropic viruses in biliary atresia. *Eur J Pediatr* 2009;168:469–76.
35. Ponsioen CY, Pannekoek Y, Vergani D, et al. Chlamydia infection as risk factor for pediatric biliary tract disease. *Eur J Gastroenterol Hepatol* 2008;20:365–6.
36. Cappello F, Conway de Macario E, Di Felice V, et al. Chlamydia trachomatis infection and anti-Hsp60 immunity: the two sides of the coin. *PLoS Pathog* 2009;5:e1000552.
37. Tiao M-M, Tsai S-S, Kuo H-W, et al. Epidemiological features of biliary atresia in Taiwan, a national study. *J Gastroenterol Hepatol* 2008;23:62–6.
38. Nosratabadi R, Bagheri V, Zare-Bidaki M, et al. Toll like receptor 4: an important molecule in recognition and induction of appropriate immune responses against Chlamydia infection. *Comp Immunol Microbiol Infect Dis* 2017;51:27–33.
39. Majumdar M, Ratho RK, Chawla Y, et al. Role of TLR gene expression and cytokine profiling in the immunopathogenesis of viral hepatitis E. *J Clin Virol* 2015;73:8–13.
40. Urcuqui-Inchima S, Cabrera J, Haenni A-L. Interplay between dengue virus and Toll-like receptors, RIG-I/MDA5 and microRNAs: implications for pathogenesis. *Antiviral Res* 2017;147:47–57.
41. Hendrickx R, Stichling N, Koelen J, et al. Innate immunity to adenovirus. *Hum Gene Ther* 2014;25:265–84.
42. Fischler B, Haglund B, Hjern A. A population-based study on the incidence and possible pre- and perinatal etiologic risk factors of biliary atresia. *J Pediatr* 2002;141:217–22.

43. Bae J-M. Interpretation of the hygiene and microflora hypothesis for allergic diseases through epigenetic epidemiology. *Epidemiol Health* 2018;40:e2018006.
44. Alexandre-Silva GM, Brito-Souza PA, Oliveira ACS, et al. The hygiene hypothesis at a glance: early exposures, immune mechanism and novel therapies. *Acta Trop* 2018;188:16–26.
45. Wang J, Qian T, Jiang J, et al. Gut microbial profile in biliary atresia: a case-control study. *J Gastroenterol Hepatol* 2019;35:334–42.
46. Hov JR, Karlsen TH. The microbiome in primary sclerosing cholangitis: current evidence and potential concepts. *Semin Liver Dis* 2017;37:314–31.
47. Tang R, Wei Y, Li Y, et al. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut* 2018;67:534–41.
48. Lee KJ, Kim JW, Moon JS, et al. Epidemiology of biliary atresia in Korea. *J Korean Med Sci* 2017;32:656–60.
49. Xavier-Carvalho C, Cardoso CC, De Souza Kehdy F, et al. Host genetics and dengue fever. *Infect Genet Evol* 2017;56:99–110.
50. Kambhampati A, Payne DC, Costantini V, et al. Host genetic susceptibility to enteric viruses: a systematic review and metaanalysis. *Clin Infect Dis* 2016;62:11–8.