

Potential Benefit of Probiotic E. Coli Nissle in Term Neonates


A Multicentre Randomised Double Blind Controlled Trial

Potentieller Nutzen des Probiotikums E. coli Nissle bei Neugeborenen

Eine multizentrische randomisierte kontrollierte Doppelblindstudie



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ABSTRACT

Background Probiotics are often viewed as an immunity enhancing agent. The objective of this study was to investigate whether oral administration of Escherichia coli Nissle 1917 reduces the number of infections, their duration, and severity in the first 24 months after parturition in healthy neonates.

Subjects and methods This prospective, confirmatory, randomised, double-blind, placebo-controlled study enrolled 567 healthy neonates from four German and two Polish sites. Neonates received 10e8 viable E. coli Nissle (n = 283) or placebo (n = 284) daily in the first week and every second day in week 2 and 3. After 6 and 12 months, the subjects received additional instillations on ten subsequent days. The overall efficacy was assessed by the number of infections per observation period.

Results Incidence rates of infection, infection duration and severity showed no statistically significant difference between groups after 24 months. Post-hoc analyses, however, revealed a short-term benefit of E. coli Nissle four weeks after treatment start which became less pronounced after eight weeks. E. coli Nissle was safe and well tolerated.

Conclusions A long-term effect after colonising the healthy neonate's gut with E. coli Nissle to protect against infections could not be shown. Additional studies are needed to confirm a transitory, yet clinically significant role of probiotics in the first four weeks after parturition.

ZUSAMMENFASSUNG

Hintergrund Probiotika werden oftmals als Immunstimulans gewertet. In dieser Studie wurde untersucht, ob sich für reife Neugeborene durch die perorale Verabreichung von *Escherichia coli* Nissle 1917 die Zahl der Infektionen, ihre Dauer und Schwere in den ersten 24 Monaten nach Geburt verringert.

Probanden und Methoden 567 gesunde Neugeborene aus vier deutschen und zwei polnischen Studienzentren wurden in diese prospektive, konfirmatorische, randomisierte, doppelblinde und Placebo-kontrollierte Studie eingeschlossen. Neugeborene erhielten 10e8 lebende *E. coli* Nissle (n = 283) oder Placebo (n = 284) täglich in der ersten Woche und jeden zweiten Tag in Woche 2 und 3. Nach 6 und 12 Monaten fanden zusätzliche Verabreichungen an zehn aufeinanderfolgenden Tagen

statt. Die Wirksamkeit insgesamt wurde anhand der Zahl der Infektionen im Beobachtungszeitraum beurteilt.

Ergebnisse Nach 24 Monaten bestand zwischen den Gruppen kein statistisch signifikanter Unterschied in den Inzidenzraten der Infektionen, Dauer oder Schwere der Infektionen. Post-hoc Analysen ergaben allerdings einen kurzfristigen Nutzen von *E. coli* Nissle vier Wochen nach Beginn der Therapie, der nach acht Wochen nachgelassen hatte. *E. coli* Nissle war sicher und wurde gut toleriert.

Schlussfolgerungen Es konnte kein gegen Infektionen schützender Langzeiteffekt nach intestinaler Besiedlung mit *E. coli* Nissle für den gesunden Neugeborenen gezeigt werden. Zusätzliche Studien sind nötig, um die vorübergehende, jedoch klinisch signifikante Rolle des Probiotikums in den ersten vier Wochen nach Entbindung zu bestätigen.

Introduction

Intestinal colonisation in the neonate is essentially important in establishing mucosal immunity [1]. The gut microbiome may be therapeutically modulated to introduce colonising species, so-called probiotics, that afford an advantage in the antibacterial and antiviral mucosal response. Because a common mucosal system exists, changes of the gut microbiome may also affect respiratory or urogenital immunity [2] and such changes may be desirable in the neonate [3].

E. coli Nissle 1917 colonises the gut [4], as demonstrated by the long-term presence (up to six months) of *E. coli* in stool of 24/27 healthy newborns [5]. A parallel group, controlled open label prospective trial in 62 late preterm newborns in which *E. coli* Nissle 1917 suspension was administered for three weeks, showed a significantly reduced incidence of acute respiratory infections during the first 28 days after parturition. This was no longer observed at six or twelve months. There was a trend for fewer acute respiratory infections, of hospitalisations and shorter duration of these during the newborn period of up to 28 days, suggesting a more sustained beneficial effect of treatment [6]. *E. coli* Nissle 1917 may be effective against uropathogens [7] and antibiotic induced, rotavirus-associated gastroenteritis [8].

Typically, *E. coli* Nissle has been tested in children and adults with underlying disease [9]. Probiotic supplementation in children aged 0–59 months has been described as safe [10] but its clinical value remains uncertain because of a lack of randomised clinical trials [11]. Importantly, breast feeding transfers maternal, gut-colonising bacteria to the suckled [12].

The purpose of this trial was to assess efficacy and safety in healthy neonates of *E. coli* Nissle administered over the neonatal period and at six and twelve months. The hypothesis was that colonising neonatal gut with *E. coli* Nissle significantly reduces the incidence of infections over 24 months.

Materials and Methods

Regulatory

The trial was conducted at four sites in Germany (Klinikum Südost Rostock, Klinikum Westbrandenburg Potsdam, Universitätsklinikum Jena, Krankenhaus St Elisabeth & St Barbara Halle) and at two sites in Poland (Oddział Kliniczny Noworodków, Wczesniaków, Bydgoszcz, and Oddział Kliniczny Neonatologii, Warszawa) from 7. October 2015 till 10. October 2020 under the auspices of Ardeypharm GmbH. After obtaining a favourable ethics vote and approval by German and Polish competent authorities, as applicable, recruitment began. Several amendments were necessary for sites in both countries and pertained to specification of criteria, addition of sites, increase of sample size, specification of procedures, COVID measures, adaptation of ICF, or change of PI. Audits were carried out in May 2017 (site audit, Rostock) and in 2018 (qualification audit, CRO). Data base was locked on 04. February 2020.

Investigational product

E. coli Nissle 1917 suspension for oral administration (10⁸ viable bacteria (CFU)/ml) was provided by Ardeypharm GmbH in several batches (533390, 613500, 633590, 713750, 733840, 813960, 834030, 844100, 924240). The placebo control was provided in an identical ampoule and had identical appearance but lacked the active ingredient (batch 922185).

Trial design

Scientific advice was sought from the Federal Institute for Drugs and Medical Devices while preparing the study concept. The trial was designed as an authorisation study (phase IIIb) and was deposited with the EudraCT number 2015-001763-39.

For the sample size calculation, average numbers of infections in the first two years after parturition of 6 under placebo, and of 4.8 under probiotic treatment were assumed, with a common standard deviation of 3.85. Randomisation of 223 participants in each group would achieve a statistical power of 90% at one-sided significance level of $\alpha = 0.025$. Assuming 20% dropouts, the estimated sample

► **Table 1** Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Signed informed consent (by both parents)	Birth weight <2000g
First intake of trial suspension within the first five days of life	APGAR scores <5 at 5 min or <8 at 10 min
Functionally mature newborn >35 th gestational week	pH <7.0 in cord blood
Mother's intention to breastfeed	Antibiotic treatment of perinatal infection
Avoidance of all other probiotic administration	Maternal TORCH infection

size was increased to 279 participants per study arm (total sample size of 558 participants). For the trial, 567 newborns were recruited according to inclusion and exclusion criteria (► **Table 1**) and randomised to one of two study arms (disposition, see ► **Fig. 1**). A permuted block randomisation strategy allowed 1:1 allocation per site. Study team and parents were blinded to the treatment, as suspensions were distributed with labels of the individual's randomisation number. Neonates received 1 ml of *E. coli* Nissle (n = 283) or placebo (n = 284) daily in the first week of life and every second day in week 2 and 3. After 6 and 12 months, additional administrations followed over ten subsequent days (overview, ► **Fig. 2**).

Assessments

Efficacy

The number of infections (respiratory, gastrointestinal, urological) per observation period was counted. All rhinitides that occurred in the first year of a child's life were considered for the count of the primary efficacy variable. Rhinitides that occurred in the second year of a child's life were only included if they were accompanied by fever (body temperature exceeding 38.4 °C during at least one measure). The duration of an episode of illness was defined as the time from the appearance of the first symptom of a study relevant infection to the disappearance of the last symptom of a study relevant infection. Study relevant infections that occurred concurrently, or one after another, within a period of 7 days were only counted separately if they belonged to different systems.

Safety and Tolerability

All adverse events that occurred during the participation of individual subjects in the trial were recorded and compared between the two groups in terms of severity, intensity, causality, and action taken, to assess the safety of *E. coli* Nissle. The acute infections defined as efficacy variables were explicitly not assessed as AEs. AEs were considered potentially trial medication-related if the causality to trial medication use was classified by the investigator as "certain", "probable" or "possible" (according to the WHO Collaborating Centre for International Drug Monitoring). In addition, the tolerability of the medication was assessed by the investigator (1 = very good, 2 = good, 3 = moderate, 4 = poor) and compared between the two groups.

Statistics

All statistical analyses were completed using SAS. Continuous variables were summarised using descriptive statistics, including num-

ber of subjects, mean, standard deviation, quartiles, median, minimum, and maximum. For categorical variables, summaries included counts of subjects and percentages.

The primary objective was the comparison of incidences of infection between the two groups in the first 24 months after parturition. The primary efficacy variable was the total number of infections (bacterial and viral) observed for each subject during their individual study participation standardised per month during the first 24 months of the infant's life. Infections were acute upper or lower respiratory tract infections, gastroenteritis, or urinary tract infections.

The secondary objectives were the comparison of severity of infection between the two groups and the tolerability of *E. coli* Nissle. Comparison of treatment groups was performed using t-test with 95% confidence interval (CI) for the following secondary efficacy variables: mean duration of the infection (numbers of days with at least one symptom), mean number of hospital admissions caused by infections, mean number of in-hospital spent days, mean number of systemic antibiotic treatments due to study relevant infections.

Primary and secondary efficacy analyses were based on the full analysis set (defined according to the intention-to-treat principle).

Safety variable was the number of adverse events. The analysis of safety was based on the safety analysis set (all subjects (as treated) who received at least one dose of trial medication).

Post-hoc analyses were conducted for the primary efficacy variable at 4 and 8 weeks.

Results

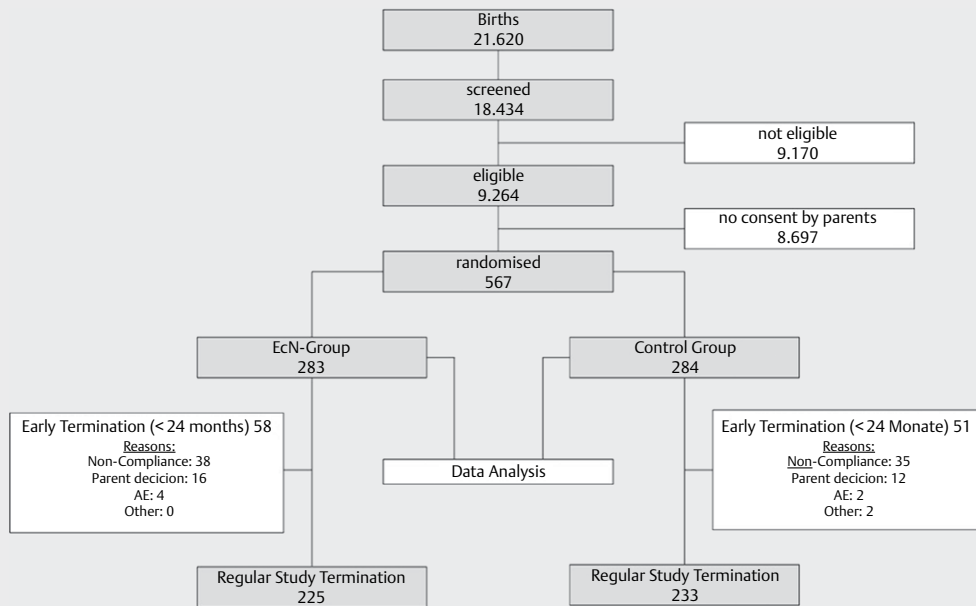
Description of cohort

There was no significant difference between the subjects randomised to the study arms in terms of gestational age, physiological parameters at birth or mode of delivery (► **Table 2**). There was a slight preponderance of male to female neonates. Comparable numbers of neonates had siblings in the same household and age at first administration of the investigational product or placebo was comparable (► **Table 2**). The doses administered over the duration of the trial were comparable between the study arms. The median dosage for all was 34 (► **Table 3**). There was no difference between the groups in duration of breast feeding or consumption of formula milk (suppl. ► **Table 1**, online).

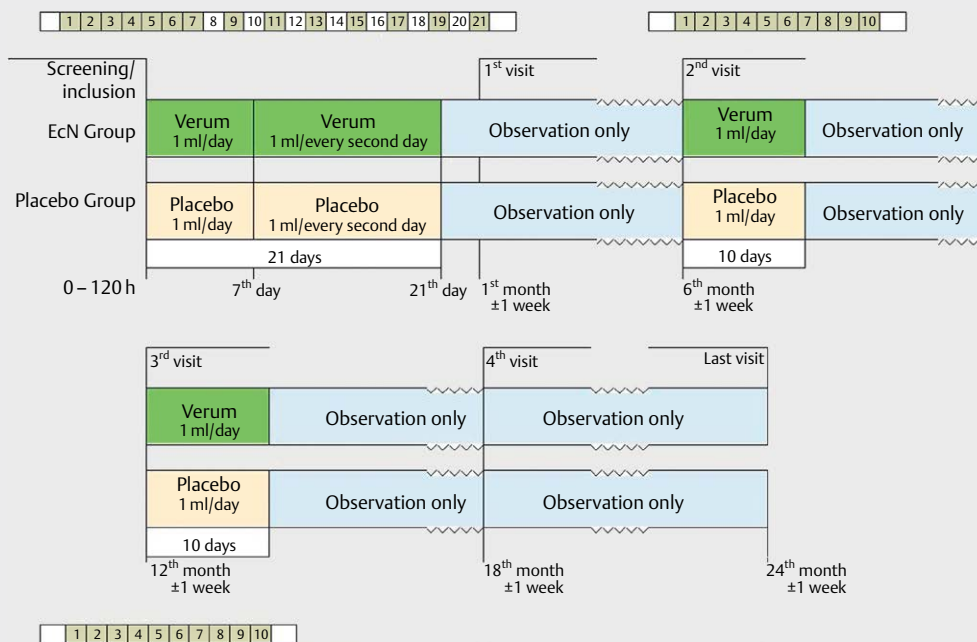
Subjects were analysed for the effect of *E. coli* Nissle on physiological (body length, weight, head circumference) and neurophysiological development as part of standard pediatric checkup appointments. There was no statistical difference between the groups for any of these parameters (data not shown).

Assessment of Efficacy

Incidence rates for infections (respiratory, gastrointestinal, urological) were similar in both study arms over the duration of the trial. There was no statistically significant difference in the confirmatory analysis (► **Table 4**). All secondary efficacy variables (mean duration of infections, mean number and durations of infection-related hospital admissions, mean number of antibiotic treatments) over the study duration showed comparable outcomes (► **Table 5**).



► **Fig. 1** Disposition of subjects. EcN, *E. coli* Nissle.



► **Fig. 2** Study design. Neonates participating in the study were treated immediately after birth during the first three weeks. In the first week, subjects received 1 ml *E. coli* Nissle (EcN) suspension (verum) or placebo (1 ml daily), followed by 1 ml every other day for the following two weeks. After 6 and 12 months, subjects were re-treated with 1 ml suspension daily for ten consecutive days. Follow-up visits were conducted at 1, 6, 12 and 18 months of age as part of routine visits.

Post-hoc analyses of neonates without infection and more than one infectious episode suggested a positive effect of *E. coli* Nissle at four weeks' administration of investigational product compared to placebo, as the incidence rate ratio *E. coli* Nissle versus placebo showed a shift from 1 (► **Table 6a**). This trend was further support-

ed by a significant difference in the distribution of subjects with and without infection in the four weeks after start of the intake phase ($\chi^2 = 9.94$; $p = 0.002$). In addition, an odds ratio of 0.58 and a relative risk reduction of 28% underline the efficacy of *E. coli* Nissle (► **Table 6a**). This finding was statistically significant (exceeding probability).

► **Table 2** Characterisation of the study population

Item		EcN (n = 283)	placebo (n = 284)
Gestational age [weeks]		39.4 ± 1.2	39.4 ± 1.3
Age at 1 st IP intake [h]		64.5 ± 22.5	63.6 ± 25.1
Sex	Male	153 (54.1)	143 (50.4)
	Female	130 (45.9)	141 (49.6)
Mode of parturition	vaginal delivery	229 (80.9)	220 (79.2)
	cesarean section	54 (19.1)	64 (20.8)
length at birth [cm]		51.5 ± 2.8	51.5 ± 2.6
weight at birth [g]		3525.4 ± 444.6	3535.5 ± 439.0
Head circumference [cm]		35.1 ± 1.7	35.1 ± 1.4
5 Min APGAR		9.6 ± 0.6	9.5 ± 0.7
10 Min APGAR		9.8 ± 0.4	9.8 ± 0.4
pH of umbilical cord blood		7.3 ± 0.1	7.3 ± 0.1
Siblings in same household		148 (52.3)	132 (49.5)

All data are means ± SD or proportions, % in brackets; EcN, *E. coli* Nissle; IP, investigational product

► **Table 3** Administered doses over the duration of the trial.

	EcN-suspension (n = 283)	placebo (n = 284)	Overall (n = 567)
n	273	276	549
Mean (SD)	31.5 (7.03)	31.3 (7.00)	31.4 (7.01)
Median	34.0	34.0	34.0
Q1, Q3	34.0, 34.0	34.0, 34.0	34.0, 34.0
Min, Max	1, 34	0, 34	0, 34

EcN, *E. coli* Nissle

The effect of the *E. coli* Nissle after eight weeks' intake was less pronounced. Incidence rates of *E. coli* Nissle and placebo treated subjects became more similar as indicated by an incidence rate ratio closer to 1 (► **Table 6b**). Nevertheless, there was still a statistically significant advantage of *E. coli* Nissle subjects in terms of proportion of children not suffering from any infection within eight weeks of starting the regimen phases ($\chi^2 = 5.84$; $p = 0.016$). Similarly, an odds ratio to experience at least one infection within eight weeks of 0.66 and a relative risk reduction of 16% pointed towards an advantage of *E. coli* Nissle subjects (► **Table 6b**). Again, this finding was statistically significant (exceeding probability).

The incidence rates were calculated for subgroups defined by the duration of breastfeeding or delivery by cesarean section for four and eight weeks of administration of the investigational product or placebo (► **Fig. 3**). There is conformity in the observation that the incidence rate ratio that was calculated for the *E. coli* Nissle group compared with the placebo group was lower than 1 when *E. coli* Nissle was administered for four weeks, and lower than the eight-week incidence rate ratio, given comparable confidence intervals for each of the subgroup pairs.

► **Table 4** Incidence rates of infections over 24 months.

	EcN-suspension (n = 283)	placebo (n = 284)
Mean subject time under observation (months) (SD)	20.22 (7.891)	20.68 (7.476)
Incidence rate	0.24	0.25
95% confidence interval (CI)	(0.22, 0.27)	(0.23, 0.27)
Incidence rate ratio EcN versus Placebo	0.99	–
95% CI for incidence rate ratio	(0.87, 1.12)	–
p-value (one-sided)	0.4227	–

EcN, *E. coli* Nissle

► **Table 5** Secondary variables over the duration of the trial.

	EcN-suspension (n = 283)	placebo (n = 284)
Mean duration of infections in days (SD)	50.2 (57.55)	50.9 (51.3)
Median	30.0	36.5
Estimate Difference of EcN-suspension versus placebo	– 0,60	–
95% Confidence Interval for Difference of EcN-suspension versus placebo	(– 9.60, 8.39)	–
Mean number of hospital admissions caused by infections (SD)	0.2 (0.44)	0.1 (0.41)
Median	0.0	0.0
Estimate Difference of EcN-suspension versus placebo	0.05	–
95% Confidence Interval for Difference of EcN-suspension versus placebo	(– 0.02, 0.12)	–
Mean number of In-hospital spent days (SD)	0.7 (2.30)	0.6 (2.29)
Median	0.0	0.0
Estimate Difference of EcN-suspension versus placebo	0.20	–
95% Confidence Interval for Difference of EcN-suspension versus placebo	(– 0.18, 0.58)	–
Mean number of systemic antibiotic treatments due to study relevant infections (SD)	0.6 (1.09)	0.6 (1.01)
Median	0.0	0.0
Estimate Difference of EcN-suspension versus placebo	0.08	–
95% Confidence Interval for Difference of EcN-suspension versus placebo	(– 0.09, 0.26)	–

EcN, *E. coli* Nissle

Assessment of Safety and Tolerability

From a total of 405 subjects, 1069 adverse events were reported. The number of subjects with at least one adverse event was similar in the two study arms (209 (73.9%) in the *E. coli* Nissle group and 196 (69.3%) in the placebo group). 33 subjects (11.7%) in the *E. coli*

► **Table 6** post-hoc analysis for primary efficacy variable

a		
4 weeks after administration of study drug	EcN-suspension (n = 283)	placebo (n = 284)
Mean subject time under observation (days) (SD)	74.44 (21.96)	75.62 (20.71)
Incidence rate	0.0068	0.0084
95 % confidence interval (CI)	(0.0057, 0.0081)	(0.0071, 0.0099)
Incidence rate ratio EcN versus placebo	0.81	–
95 % CI for incidence rate ratio	(0.64, 1.03)	–
p-value (one-sided)	0.0445	
Odds Ratio (Relative Risk Reduction)	0.5788 (0.28)	
Odds Ratio p-value (2-sided)	0.0017	
Odds Ratio 95 % CI	0.4114, 0.8141	
b		
8 weeks after administration of study drug	EcN-suspension (n = 283)	placebo (n = 284)
Mean subject time under observation (days) (SD)	147.34(47.575)	150.14(44.224)
Incidence rate	0.0071	0.0080
95 % confidence interval (CI)	(0.0063, 0.0082)	(0.0071, 0.0091)
Incidence rate ratio EcN versus placebo	0.89	–
95 % CI for incidence rate ratio	(0.74, 1.07)	–
p-value (one-sided)	0.1096	
Odds Ratio (Relative Risk Reduction)	0.6622 (0.16)	
Odds Ratio p-value (2-sided)	0.0159	
Odds Ratio 95 % CI	0.4736, 0.9257	
EcN, <i>E. coli</i> Nissle ; EcN, <i>E. coli</i> Nissle		

Nissle group and 23 (8.1 %) in the placebo group reported at least one SAE. The majority was related to infection and gastrointestinal events (*E. coli* Nissle: 11, Placebo: 8). There were no obvious differences between the treatment groups. No adverse event resulted in death during the study. There were 18 subjects (6.4 %) in the *E. coli* Nissle group (placebo: 19, 6.7 %) with at least one potentially drug-related AE (multiple counts possible); none of them was serious.

Most investigators reported a good or very good tolerance of the trial medication. Overall, investigators reported only 12 subjects in the *E. coli* Nissle group and 22 subjects (measured at three occasions, multiple counts possible) in the placebo group showing moderate or poor tolerability.

Discussion

The use of probiotics as medicinal products requires GMP conform production and evidence of their therapeutic benefit in the population of interest [13]. This study investigated efficacy and safety of *E. coli* Nissle suspension in a randomised controlled trial of term newborns. The hypothesis that colonisation of the healthy neonate

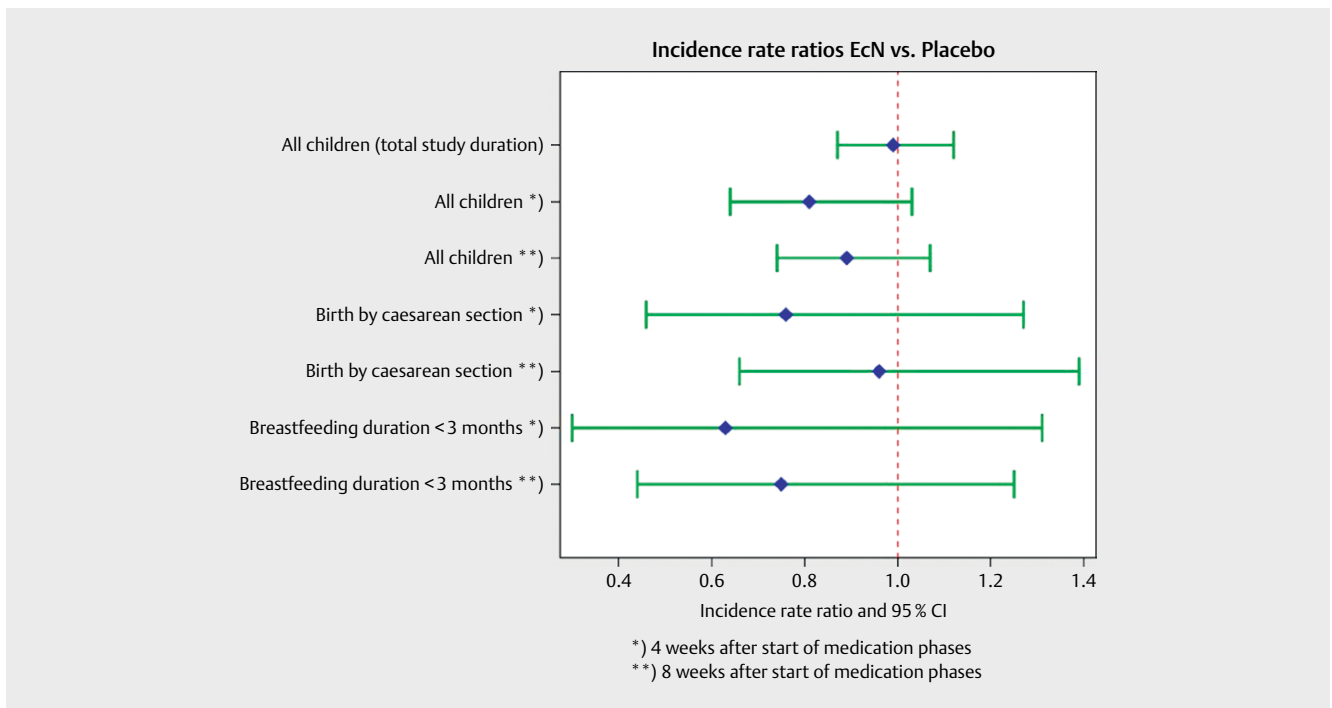
with *E. coli* Nissle would lead to a sustainable benefit in the reduction of infections was rejected. However, post-hoc analyses revealed a significant effect at four weeks.

Duration of breastfeeding is recommended by the WHO to be six months [14]. Its potential role, however, in decreasing the incidence of respiratory and gastrointestinal tract infections compared to three or four months of breastfeeding is derived from observational studies only in developed countries [15]. Delivery by caesarean section was found associated with greater respiratory morbidity (defined as transient tachypnea neonatal, respiratory distress syndrome, or persistence pulmonary hypertension) than at-term vaginal delivery [16]. These two variables were analysed in our study's post-hoc analyses, in addition to early timepoints (prior to six months). Whilst the subgroup analyses of infants who were breastfed for less than three months and those delivered by caesarean section (► **Fig. 3**) did not show significant differences between the study arms, taken together, they do underscore a positive effect of *E. coli* Nissle, administered for four weeks. This benefit was most pronounced when analysing the incidence rate of infections in infants over a four-week duration: there was a significant difference between the two study arms with a relative risk reduction of 28 %, meaning that the inherent risk to contract an infection as term neonate was lowered.

The first two years are thought to be crucial for the development of the symbiotic microbiome on mucosal surfaces that is relevant in the maintenance of health and development of disease [17]. Microbial diversity in the neonatal gut is influenced by breast or formula feeding but early differences are lost at one year of age [18]. It is conceivable that a window of opportunity exists in which colonisation the neonatal gut is most effective. This hypothesis may be supported by a placebo controlled double blind randomised trial of 290 infants (aged 8-14 months) which failed to show a benefit of daily probiotic administration (for six months) in reducing infection-related absences from childcare provision [19].

Few studies of *E. coli* Nissle in human, term neonates exist. A multicenter observational study in primary care practices studied the efficacy and safety of *E. coli* Nissle in 668 pediatric subjects, of whom 163 were neonates and infants aged less than two years, over twelve weeks. Gastrointestinal ailments, increased infection and atopy were indications that were successfully and safely treated [20]. *E. coli* Nissle was administered in a double blind study to term neonates within the first week after birth and was found to colonise the gut and outcompete potential pathogens [5]. A confirmative double blind study in 113 infants and toddlers (age range 2–47 months) suffering from acute diarrhoea showed a greater and quicker response rate in those treated with *E. coli* Nissle than those who received placebo [21]. Efficacy and safety were reproduced in another double-blind outpatient study of 151 infants and toddlers (age range 1–47 months) who presented with diarrhoea lasting at least 4 days, but less than a fortnight and were well nourished [22].

A recent systematic review found that probiotics were safe to administer perinatally and in infancy [23]. Our randomised controlled trial shows that *E. coli* Nissle was safe and well tolerated. There was no difference between *E. coli* Nissle and placebo that could be concluded from the reported adverse events nor from investigators' assessments of tolerability.



► **Fig. 3** Incidence rate ratios and 95% confidence intervals (CI) for *E. coli* Nissle (EcN) vs placebo groups

Plasticity in microbial gut community is greatest in the first four weeks after parturition when significant differences between modes of delivery exist and breast milk matures from colostrum rich in secretory IgA [24]. By eight weeks, breast feeding equalises differences seen in microbial compositions after vaginal delivery or cesarean section. At 12 months, environmental determinants have gained greater importance in shaping the gut microbiome [25]. Hence, our analyses of the full analysis set of our healthy trial subjects may support the presence of a developmental timeline in gut colonisation that can be efficaciously used when administering pediatric probiotics, especially *E. coli* Nissle.

Contributor's Statement

D. Olbertz, M. Radke, C. Wolff conceived and designed the study. D. Olbertz led the drug study as Head of Clinical Trial. M. Radke acted as Consultant of Ardeypharm GmbH in the development of the study and discussions with the higher federal authority; C. Wolff acted as project manager of the ongoing study. D. Olbertz, H. Proqiuutté, L. Patzer, T. Erler, A. Mikolajczak, I. Sadowska-Krawczenko were the lead investigators at the recruiting sites, collected data and revised the manuscript.

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Conflict of Interest

D. Olbertz received an investigator's fee and reimbursements for two related congress presentations and associated travel from Ardeypharm GmbH; C. Wolff was employed by Ardeypharm GmbH for the duration of the study; M. Radke received consultancy fees in relation to the project from Ardeypharm GmbH. All authors received reimbursement of study-related expenditures from Ardeypharm GmbH.

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Notice

This article was changed according to the following Erratum on December 13th 2022.

Erratum

In the above mentioned article the first sentence of the paragraph “Regulatory” was wrong correct is:

The trial was conducted at four sites in Germany (Klinikum Südstadt Rostock, Klinikum Westbrandenburg Potsdam, Universitätsklinikum Jena, Krankenhaus St Elisabeth & St Barbara Halle) and at two sites in Poland (Oddział Kliniczny Noworodków, Wczesniaków, Bydgoszcz, and Oddział Kliniczny Neonatologii, Warszawa) from 7. October 2015 till 10. October 2020 under the auspices of Ardeypharm GmbH