

# Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey

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## Summary

**Background** A farming environment protects against development of asthma, hay fever, and atopic sensitisation in children. We aimed to establish whether increased exposure to microbial compounds has to occur early in life to affect maturation of the immune system and thereby reduces risk for development of allergic diseases.

**Methods** We did a cross-sectional survey in rural areas of Austria, Germany, and Switzerland. 2618 (75%) of 3504 parents of 6–13-year-old children completed a standardised questionnaire on asthma, hay fever, and atopic eczema. Children from farming families, and a random sample of non-farmers' children, who gave consent for blood samples to be obtained for measurements of specific serum IgE antibodies to common allergens were invited to participate (n=901).

**Findings** Exposure of children younger than 1 year, compared with those aged 1–5 years, to stables and consumption of farm milk was associated with lower frequencies of asthma (1% [3/218] vs 11% [15/138]), hay fever (3% [7] vs 13% [18]), and atopic sensitisation (12% [27] vs 29% [40]). Protection against development of asthma was independent from effect on atopic sensitisation. Continual long-term exposure to stables until age 5 years was associated with the lowest frequencies of asthma (0.8% [1/122]), hay fever (0.8% [1]), and atopic sensitisation (8.2% [10]).

**Interpretation** Long-term and early-life exposure to stables and farm milk induces a strong protective effect against development of asthma, hay fever, and atopic sensitisation.

*Lancet* 2001; 358: 1129–33

## Introduction

Growing up on a farm protects against allergic sensitisation and development of childhood allergic diseases.<sup>1,2</sup> Regular contact with farm animals confers an important protective effect in such an environment.<sup>2,3</sup> We have noted<sup>4</sup> higher concentrations of endotoxin (lipopolysaccharide, derived from cell walls of gram-negative bacteria) in dust from kitchen floors and children's mattresses in farming families than in non-farming families. Endotoxin and other microbial compounds regulate various processes in the immune system, such as production of interleukin 12 and interferon gamma. These factors select against T-helper-2 cells and, thus, counteract allergic sensitisation.<sup>7–10</sup> The predominant type of response (T-helper-1-like or T-helper-2-like) to a specific antigen is determined at the first encounter with the antigen. In early life, the T-helper-2-polarisation of the fetal immune system is progressively replaced by T-helper-1-dominance.<sup>11,12</sup> Thus, microbial burden in the first years of life could be crucial for development of a non-atopic immune response. We aimed to establish whether the timing of exposure to a farming environment affects the protection such exposure confers from development of asthma, hay fever, and allergic sensitisation.

## Methods

### Participants

We did a cross-sectional survey in rural areas of Austria, Germany, and Switzerland. All these regions are fairly similar with respect to population density and farming characteristics; most farms are small and run by family members and only rarely by farm workers. 98% of these farmers own cattle, pigs, sheep, poultry, horses, or goats.<sup>2</sup> We did fieldwork between March and July, 1999. We invited parents of 3504 children in school grades 1–6 to answer a questionnaire on respiratory and allergic diseases, which included ISAAC (international study of asthma and allergies in childhood) core questions<sup>13</sup> and questions on environmental factors.

2618 (75%) parents participated and were asked to give consent to further investigations that included measurement of specific IgE (RAST) in serum, and collection of dust samples. 1406 (54%) of these 2618 families gave consent. All children from farming families and a random sample of children from non-farming families from the same rural area were invited to participate (n=901). We restricted our final analysis to children born in Austria, Germany, or Switzerland to parents who were nationals of those countries (n=812) to avoid confounding by ethnicity.

Approval for the survey was obtained from the three local ethics committees for human studies and from the principals of the schools involved. Informed written consent was obtained from all parents.

### Procedures

From the questionnaires we recorded the frequency of diseases and symptoms, and assessed potential

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	Farmers (n=319)	Non-farmers (n=493)
<b>First visit to stable</b>		
In 1st year of life	63% (201)	16% (77)
At 1-4 years	29% (94)	30% (149)
<b>Time spent in the stable in the 1st year of life (min daily)</b>		
None	37% (118)	84% (415)
1-10	9% (30)	8% (39)
11-20	15% (49)	4% (19)
21-60	26% (84)	3% (16)
>60	12% (38)	1% (4)
<b>Mother active on the farm during pregnancy</b>		
No	18% (56)	..
Less than once per week	10% (31)	..
1-6 times per week	17% (53)	..
Daily	20% (64)	..
>once daily	36% (114)	..
<b>Consumption of farm milk in 1st year of life</b>	82% (260)	35% (171)
<b>Consumption of farm milk by pregnant mother</b>	80% (255)	33% (161)

Table 1: Exposure to characteristics of a farming environment in farmers' children and children not living on a farm

explanatory and confounding factors. Children whose parents reported a doctor's diagnosis of asthma, or recurrent asthmatic, obstructive, or spastic bronchitis were classed as having asthma. If parents reported at least one attack of wheeze during the past 12 months we classified children as having symptoms of asthma. Atopic dermatitis and hay fever were defined as parents' report of a doctor's diagnosis of atopic dermatitis and hay fever. Symptoms of hay fever were defined as a positive response to: "in the last 12 months, has your child had problems with sneezing or a runny or blocked nose without a cold accompanied by itchy-watery eyes?" We recorded a positive response to: "has your child ever had an itchy rash which was coming and going for at least 6 months?" as indicative of symptoms of eczema. Farmers' children were defined as children whose parents answered yes to the question "does your child live on a farm?"

Centrally-trained fieldworkers did another interview with farming and control parents to ascertain details of timing, frequency, and intensity of children's exposure to stables and farm and pet animals; mothers' activity on the farm; duration of breastfeeding; timing of consumption of home-grown food and farm milk; vaccinations; and avoidance of allergens.

All serum IgE measurements were done at a central laboratory (Charité; Berlin, Germany). The serum concentration of specific IgE raised against a panel of aeroallergens (mixed-grass pollen, birch pollen, mugwort

pollen, *Dermatophagoides pteronyssinus*, cat dander, dog dander, and *Cladosporium herbarum*) and a panel of food allergens (egg white, milk protein, cod fish, wheat flour, peanut, and soybean) was measured in all samples by fluorescence enzyme immunoassay (SX1 and FX5 CAP, Pharmacia; Uppsala, Sweden). We also measured concentrations of specific IgE antibodies to cow epithelium and storage mites (*Lepidoglyphus destructor*) in all serum samples (CAP, Pharmacia; Uppsala, Sweden). In children with a positive SX1 test result, responses to specific allergens (grass pollen, birch pollen, *Dermatophagoides pteronyssinus*, and cat dander) were measured. Likewise, in children with a positive FX5 test result, responses to egg white and milk protein were measured. We defined atopic sensitisation as at least one positive specific IgE test result of 3.5 kU/L or greater for the six aeroallergens (house dust mite, storage mite, grass and birch pollen, cat dander, and cow epithelium).

#### Statistical analyses

Data entry and analyses were done with SPSS for Windows versions 6.0, 8.0, and 10.0. The frequencies of symptoms, diagnoses, and allergic sensitisation were compared between children living on farms and non-farming families by  $\chi^2$  test or Fisher's exact test, as appropriate. Adjusted odds ratios were calculated by fitting logistic regression models. Adjustment was made for potential confounders such as age, sex, study area, family history of asthma and hay fever, education of parents, and number of older siblings. Breastfeeding and passive smoking were also included, but had no significant effect, and therefore no further adjustment for these variables was made.

To assess the effect of timing of exposure to farming environmental factors, dummy variables were created for exposure to stables in the first year of life, and for exposure to stables at ages 1-4 years but not during the first year of life, with exposure at older age or no exposure to stables used as a reference category. Likewise, dummy variables were calculated for consumption of farm milk in these age groups. Individual variables and combinations of variables were then included in a multivariate logistic regression model. We also adjusted models for farming status.

The time a child had spent in a stable in their first year and between ages 1 and 4 years was used to divide children into high and low exposure groups in each of these age categories, with the median exposure time as the cutoff. Combinations of these exposure categories were used in a multivariate model to evaluate the effect of exposure that continued after age 1 year.

	Stables and farm milk in the 1st year of life (n=218)	Stables, but no farm milk in the 1st year of life (n=48)	Farm milk, but no stables in the 1st year of life (n=189)	Farm milk, stables, or both after the 1st year of life (n=138)	Neither stables nor farm milk exposure (n=170)
<b>Asthma diagnosis</b>	1% (3) 0.14 (0.04-0.48)	6% (3) 0.51 (0.14-1.86)	6% (11) 0.48 (0.21-1.1)	11% (15) 0.88 (0.42-1.86)	12% (20) Reference
<b>At least one wheeze attack in past 12 months</b>	3% (6) 0.17 (0.07-0.45)	6% (3) 0.43 (0.12-1.52)	6% (12) 0.43 (0.20-0.92)	9% (12) 0.60 (0.28-1.28)	15% (25) Reference
<b>Hay fever</b>	3% (7) 0.20 (0.08-0.50)	4% (2) 0.25 (0.05-1.13)	4% (8) 0.24 (0.10-0.56)	13% (18) 0.88 (0.44-1.74)	16% (27) Reference
<b>Runny nose and itchy eyes in past 12 months</b>	5% (11) 0.27 (0.13-0.57)	8% (4) 0.44 (0.14-1.37)	7% (14) 0.42 (0.21-0.86)	12% (16) 0.65 (0.33-1.30)	20% (34) Reference
<b>Atopic sensitisation†</b>	12% (27) 0.32 (0.17-0.62)	21% (10) 0.56 (0.25-1.27)	15% (29) 0.43 (0.24-0.77)	29% (40) 0.99 (0.58-1.69)	33% (56) Reference

\*Adjusted for age, sex, study area, parental education, family history of asthma and hay fever, number of older siblings and farming status. †Any reaction to inhaled allergens (house dust and storage mites, cat dander, grass and birch pollen, cow epithelium) of  $\geq 3.5$  kU/L.

Table 2: Frequency and risk (adjusted\* odds ratio, 95% CI) of asthma, hay fever, and atopic sensitisation in relation to exposure to stables and farm milk in the 1st year of life

	Pregnant mother active daily on farm (n=119)	Pregnant mother not active daily on farm (n=58)	Pregnant mother not active daily on farm and child not exposed to stable and farm milk in 1st year (n=28)
Asthma diagnosis	0% ..	2% (1) 0.02 (0.001-0.47)	14% (4) Reference
At least one wheeze attack in past 12 months	1% (1) 0.04 (0.001-1.30)	3% (2) 0.17 (0.01-2.51)	7% (2) Reference
Hay fever	1% (1) 0.07 (0.005-0.91)	7% (4) 0.43 (0.06-2.87)	11% (3) Reference
Runny nose and itchy eyes in past 12 months	1% (1) 0.04 (0.003-0.53)	12% (7) 0.61 (0.13-2.87)	14% (4) Reference
Atopic sensitisation†	8% (10) 0.18 (0.06-0.56)	19% (11) 0.36 (0.12-1.08)	39% (11) Reference

\*Adjusted for age, sex, study area, parental education, family history of asthma and hay fever, and number of older siblings. †Any reaction to inhalent allergens (house dust and storage mites, cat dander, grass and birch pollen, cow epithelium) of  $\geq 3.5$  kU/L.

Table 3: Frequency and risk (adjusted\* odds ratio, 95% CI) of asthma, hay fever, and atopic sensitisation in relation to farming activity of pregnant mothers in farmers' children exposed to stables and farm milk in the 1st year of life

## Results

Complete data were available for 812 children: 319 farmers' children with mean age 9.42 years (SD 1.63), and 493 children from non-farming families, mean age 9.49 years (1.60). 418 (51%) of all children were boys.

The odds ratios for asthma and hay fever symptoms in relation to farming status did not differ significantly between the subsample of 812 eligible children whose parents had given consent to further investigations and the total group of 2618 children whose parents had answered the questionnaire (0.47 vs 0.48 in both the farming and non-farming populations, and 0.35 vs 0.43, respectively). However, slightly more children whose parents agreed to blood testing had allergic symptoms than those whose parents refused consent. We also compared exposure variables such as farm-milk consumption, time spent in a stable, and pregnant mothers' farming activity in the subsample with the whole population. In the farming population, none of the goodness-of-fit tests were significant—ie, there was no deviation from representativity. For non-farmers' children, the variable of time spent in a stable was significantly higher in the subsample of 812 children ( $p=0.0001$ ) than the whole population. Because we randomly selected farmers' and non-farmers' children from schools, our study group was representative of rural populations in Austria, Switzerland, and Germany.

The prevalence of asthma and hay fever symptoms was

significantly lower in farm children than in those from a non-farming environment (adjusted odds ratio 0.30, 95% CI 0.15-0.61; and 0.43, 0.24-0.77; respectively). Frequency of eczema did not differ between the groups. Children living on farms were also significantly less likely to be atopic than non-farming children (0.61, 0.41-0.92). The difference between farming and non-farming groups was most pronounced for sensitisation to grass pollen (0.46, 0.28-0.74). The farming population had a higher proportion of children sensitised against farm allergens than did non-farming families, but the rates of these sensitisations were low: cow epithelium 2% (6/319) vs 0.4% (2/493); and storage mites 3% (8) vs 0.2% (1); respectively. The frequency of allergic sensitisation against egg white and milk was also low, with no difference between farming and non-farming groups (0.3% vs [1/319] 0% [0/492], respectively, for both food allergens).

Exposure to farming environmental factors was not restricted to farmers' children (table 1). Many children had been exposed to most of these environmental factors in their first year of life. Substantial protection against development of asthma, hay fever, and allergic sensitisation was seen only in children exposed to stables, farm milk, or both in their first year of life (table 2). These protective factors seemed to have additive properties, since the lowest frequencies of diseases were found in children who had been exposed to both factors in their first year of life. Both exposures had independent

	Above average† exposure in 1st year of life (n=133)	Below average‡ exposure in 1st year of life (n=131)	High-high§ exposures in years 1 and 2-5 (n=122)	Low-high¶ exposures in years 1 and 2-5 (n=43)	Low-low‡ exposures in years 1 and 2-5 (n=88)	No exposure to stables in the first 5 years of life (n=267)
Asthma diagnosis	2% (3) 0.39 (0.08-1.81)	2% (3) 0.29 (0.08-1.10)	1% (1) 0.09 (0.01-0.75)	0% ..	3% (3) 0.32 (0.09-1.16)	9% (25) Reference
At least one wheeze attack in past 12 months	3% (4) 0.88 (0.22-3.51)	4% (5) 0.54 (0.19-1.53)	2% (2) 0.22 (0.04-1.05)	0% ..	6% (5) 0.52 (0.19-1.43)	12% (32) Reference
Hay fever	1% (1) 0.05 (0.01-0.46)	6% (8) 0.52 (0.19-1.45)	1% (1) 0.09 (0.01-0.74)	2% (1) 0.31 (0.04-2.60)	8% (7) 0.88 (0.35-2.21)	11% (30) Reference
Runny nose and itchy eyes in past 12 months	2% (3) 0.13 (0.03-0.56)	9% (12) 0.72 (0.31-1.68)	2% (2) 0.12 (0.03-0.59)	5% (2) 0.46 (0.09-2.27)	11% (10) 1.06 (0.47-2.39)	14% (38) Reference
Atopic sensitisation**	8% (11) 0.28 (0.12-0.66)	20% (26) 0.79 (0.43-1.48)	8% (10) 0.23 (0.10-0.53)	12% (5) 0.37 (0.12-1.08)	24% (21) 0.93 (0.50-1.73)	27% (71) Reference

\*Adjusted for age, sex, study area, parental education, family history of asthma and hay fever, number of older siblings, and farming status. Results for 11 children with above median of total duration of time spent in the stables in the 1st year of life and below median of total duration of time spent in the stables between ages 1-4 years. Total duration of time spent in stables: †above median in 1st year of life; ‡below median in 1st year of life; §above median aged <5 years; ¶below median in 1st year of life and above median at 1-4 years; ‡below median aged <5 years. \*\*Any reaction to inhalent allergens (house dust and storage mites, cat dander, grass and birch pollen, cow epithelium) of  $\geq 3.5$  kU/L.

Table 4: Frequency and risk (adjusted\* odds ratio 95% CI) of asthma, hay fever and atopic sensitisation in relation to amount and duration of exposure to stables

effects in a multivariate logistic regression model (data not shown).

Two-thirds of mothers of infants, who were exposed to stables in their first year of life, were active on the farm every day during pregnancy. Comparison of frequencies of atopic outcomes in farmers' children, who had all been exposed to stables and farm milk in their first year of life, with respect to their mother's activity on the farm indicated that prenatal exposures had a substantial protective effect (table 3).

Compared with children who had no exposure to stables in their first 5 years of life, the frequencies of hay fever and atopic sensitisation were lowest among the 133 infants who spent more than 20 minutes (median) in the stables in the first year of life, and intermediate in the 131 who had spent less than 20 minutes in the stables at that age (table 4). No dose-response relation was seen for asthma variables.

Protection was also related to continuing exposure after the first year of life. Above median exposure up to the 5th year of life (high-high exposure in table 4) was associated with the lowest frequencies of asthma, hay fever, and atopic sensitisation.

Protection from asthma was independent of state of sensitisation. In non-atopic children, the frequency of intrinsic (ie, no allergic trigger) asthma was 1% (3/241) in those who frequently visited stables in the first year of life, compared with 4% (17/399) in those who did not ( $p=0.034$ ). In sensitised children, the respective frequencies were 8% (3/37) compared with 25% (33/134);  $p=0.029$ .

Immunisation rates (BCG, diphtheria, tetanus, pertussis, measles, mumps, rubella, *Haemophilus influenzae* B, and poliomyelitis) did not differ between farmers' and non-farmers' children. Rates were generally high in both groups of children: more than 95% for diphtheria, tetanus, measles, mumps, and poliomyelitis. 83% of non-farmers' children and 90% of farmers' children had had more than six different immunisations.

## Discussion

Our results accord with findings of a lower frequency of asthma, hay fever, and atopic sensitisation in children growing up on a farm. The timing of exposure to farm characteristics in, or even before, the first year of life, and amount and duration of exposure from the first to the fifth year of life are crucial for this protective effect. An inverse relation of exposure with asthma was independent of the state of allergic sensitisation.

The mechanism by which time spent in a stable and consumption of farm milk protect against development of asthma and atopic sensitisation is not known. These factors could be surrogate markers of the microbial environment such as endotoxin, other bacterial-wall components, or bacterial DNA that is rich in non-methylated CpG dinucleotides that modulate innate immune responses. Such factors can activate antigen-presenting cells and give rise to a T-helper-1-biased immune response by the production of tumour necrosis factor  $\alpha$ , interferon gamma, interleukin 12, and interleukin 18.<sup>7,8,14,15</sup> Individuals who have developed an efficient T-helper-1 response to an allergen have low susceptibility to development of allergic reactions. Farm children are exposed to higher inhaled concentrations of endotoxin in stables and in dust from kitchens and mattresses than non-farming children.<sup>5</sup> However, swallowing could be another route of exposure to bacterial products. This theory is lent support by the independent protective effect on atopic sensitisation of

farm milk consumption and exposure to stables in the first year of life. Farm milk, which is usually raw, contains more gram-negative bacteria<sup>16</sup> and thus lipopolysaccharide,<sup>17</sup> than pasteurised milk. Therefore, the protective factor associated with consumption of farm milk could be associated with ingestion of non-infectious microbial components, with resultant changes to the commensal gut flora, or both.

Farming environments have been shown to provide more consistent protection from hay fever and allergic sensitisation than from asthma.<sup>18</sup> Exposure to a farming environment could lead to a decreased rate of allergic sensitisation and, thus, to protection against development of asthma. However, in our study, time spent in a stable in the first year of life was protective against asthma, independently of allergic sensitisation. Thus, the protective factor associated with exposure to stables in the first year of life might affect an underlying mechanism that results in both asthma and atopic sensitisation.

Tulic and colleagues<sup>10</sup> exposed rats to aerosolised lipopolysaccharide 1 day before, and 1, 4, 6, 8, and 10 days after intraperitoneal sensitisation with ovalbumin. They showed that one exposure to lipopolysaccharide, up to 4 days after sensitisation, protected against production of specific IgE, bronchial hyperresponsiveness, and inflammation in bronchoalveolar lavage fluid. However, after day 4 of sensitisation, lipopolysaccharide inhalation had detrimental effects and led to increases in allergic responses. Similar work<sup>19</sup> was done in germ-free mice; reconstitution of intestinal flora with bifidobacterium restored susceptibility of the T-helper-2-cell response to oral-tolerance induction, but only if mice were exposed to this microbe as neonates, rather than later in life. These results suggest that early exposure to environmental microbial matter might determine the overall immune status of a mammal and render it less susceptible to development of T-helper-2 reactions, such as asthma and hay fever. Our findings are also in accord with results of two epidemiological surveys:<sup>20,21</sup> children who attended day-care in the first 6-12 months of life, or who had older siblings at home were at low risk of allergic sensitisation and asthma symptoms later in life. Exposure when older than 12 months did not confer protection.

Furthermore, amount and duration of exposure also seemed to play an important part in conferring protection against development of hay fever and allergic sensitisation. For asthma, even short exposure times conferred protection. Children whose mothers were heavily involved in farming activities in pregnancy, and who themselves frequently visited stables in their first year of life, later developed much less hay fever and became less sensitised than children whose mothers were not. Thus, sensitisation could start in pregnancy, or a maternal factor related to exposure could contribute to an infant's protection.

Family history is an important risk factor for atopic sensitisation and allergic diseases in children.<sup>22</sup> We noted an association between family history and asthma and hay fever in univariate analyses, and, thus, adjusted all multivariate models for these factors. Since we did not obtain blood samples from parents, we could not adjust for atopic sensitisation. There was no difference in frequency of asthma or hay fever between pregnant mothers who actively participated in farming activities and those who did not. Furthermore, we have previously shown that children who did not grow up on a farm but who had regular contact with farm animals had low frequencies of allergic diseases and atopic sensitisation similar to those of farmers' children.<sup>2</sup> Thus, genetic

background probably does not explain the protective effect of early exposure to farming on development of asthma and allergy.

A potential limitation of our study is that we retrospectively assessed exposures in the first year of life, which might have resulted in recall bias. We think, however, that most parents are likely to remember whether they took their baby to visit stables and animals. Since an infant cannot visit these places unassisted, parents would probably remember these trips. In these communities, most parents have to take their babies to work if no-one else looks after their child. In any case, non-differential misclassification of exposure would have occurred, which would have biased results towards no protective effect. Furthermore, estimation of duration of exposure in the first year of life is probably valid, since this amount is strongly dependent on the type of activities a parent does on the farm, which in these traditional communities usually remains the same over many years.

A second limitation of our study is that we measured an objective marker for hay fever (allergic sensitisation to pollen) but not for asthma. However, we included ISAAC<sup>13</sup> core questions on asthma symptoms and asthma diagnosis. These questions have been validated against bronchial hyperresponsiveness and show a high specificity for diagnosis of asthma (around 90%).<sup>23</sup> Additionally, the German questions on asthma used in this survey have been validated against bronchial hyperresponsiveness to hypertonic saline and have also shown around 90% accuracy in diagnosis.<sup>24</sup> Therefore, we feel confident that our questions about asthma were sufficient, especially because all effects were consistently seen for both asthma diagnosis and asthma symptoms. Our findings could help to improve understanding of the origins of asthma and allergy and lead to development of prevention strategies.

#### Contributors

Josef Riedler, Charlotte Braun-Fahrlander, and Erika von Mutius designed the study, did analyses, and wrote the manuscript. Waltraud Eder, Soyoun Maisch, and Marco Waser participated in design and analyses, and did fieldwork. Mynda Schreuer and David Carr participated in design, and did statistical analyses. Rudi Schierl participated in design, fieldwork, and laboratory analyses. Dennis Nowak participated in design, analyses, and manuscript writing. The members of the ALEX Study Team participated in discussions about design and analyses and contributed to writing the report as did all authors.

#### Members of ALEX Study Team

We thank the collaborators of the ALEX Study Team: Albrecht Bufe (Bochum, Germany), Udo Herz (Marburg, Germany), Otto Holst (Borstel, Germany), Gerd Oberfeld (Salzburg, Austria), Roger Lauener (Zürich, Switzerland), Harald Renz (Marburg, Germany), and Felix Sennhauser (Zürich, Switzerland).

#### Acknowledgments

This study was supported by an Austrian FWF grant (14015-Med); a grant from Propter Hominis, Liechtenstein; by the Zurich Lung Association; by a grant from UBS, Switzerland; and by the Bavarian Ministry for the Environment.

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