Prevalence and risk factors for scrub typhus in South India

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Abstract

OBJECTIVE To determine the prevalence and risk factors of scrub typhus in Tamil Nadu, South India.
METHODS We performed a clustered seroprevalence study of the areas around Vellore. All participants completed a risk factor survey, with seropositive and seronegative participants acting as cases and controls, respectively, in a risk factor analysis. After univariate analysis, variables found to be significant underwent multivariate analysis.
RESULTS Of 721 people participating in this study, 31.8% tested seropositive. By univariate analysis, after accounting for clustering, having a house that was clustered with other houses, having a fewer rooms in a house, having fewer people living in a household, defecating outside, female sex, age >60 years, shorter height, lower weight, smaller body mass index and smaller mid-upper arm circumference were found to be significantly associated with seropositivity. After multivariate regression modelling, living in a house clustered with other houses, female sex and age >60 years were significantly associated with scrub typhus exposure.
CONCLUSIONS Overall, scrub typhus is much more common than previously thought. Previously described individual environmental and habitual risk factors seem to have less importance in South India, perhaps because of the overall scrub typhus-conducive nature of the environment in this region.

keywords scrub typhus, prevalence, risk factors, South India

Introduction

Scrub typhus is an acute febrile illness caused by the bacterium *Orientia tsutsugamushi*, which is endemic to the 'tsutsugamushi triangle', extending from South and East Asia, to the Asian Pacific Rim, to Northern Australia. Once transmitted to humans through the painless bite of the larval trombiculid mite, or chigger, this organism can cause a disseminated infection with protean manifestations. Mortality without prompt treatment can vary significantly with prior studies showing rates as high as 70% but a median rate of 6% [1]. Even when appropriate antibiotic therapy is given, in-hospital mortality reaches 9% in South India [2]. Recognised as a distinct clinical entity by the Japanese in 1810, scrub typhus was found to be a significant cause of morbidity and mortality during both World War II and the Vietnam conflict [3, 4]. For reasons that are unclear, the recorded incidence of this infection dropped off for several decades, with some areas of previous endemicity not documenting a single case after World War II [5]. However, in recent decades, scrub typhus has re-emerged. In 1986, Korea recorded its first cases of scrub typhus in decades and has seen thousands more every year since [6, 7]. That same year, the first cases were described in northern China, when it had previously only been described in the south [8]. The re-emergence has now been well described in multiple other areas, including South India in the early 2000s [9]. The report that detailed the re-emergence in South India estimated its prevalence to be around 5% [9]. However, that same report also found that scrub typhus accounted for up to 50% of all hospital admission for undifferentiated fever in that area during the cooler seasons [9]. No subsequent studies of prevalence have been performed in South India.

In addition to lacking recent prevalence data, the risk factors associated with this disease have not been investigated in South India. While some risk factors associated with the acquisition of this infection remain generally consistent across endemic areas, classically working in agriculture, studies even differ on the particulars of this [10–12]. Factors which bring mites closer to people seem to play a role. Studies from China and Indochina suggest that a closer proximity of a person's home to the edge of a village, scrub vegetation, ditches or wood piles may increase the risk of infection [13,14]. Close proximity to animals, both domestic and rodents, may also play a role [14, 15]. Poverty has been associated with this disease as well, with some studies suggesting that crowded conditions may play a role and others that it is poor household sanitation [13–15]. Personal habits, such as squatting to toilet outdoors, not bathing or changing clothing after work, not using a mat when sitting on grass to eat and even not going home from work to eat meals, have been linked with this infection [11, 16]. Wearing clothing that cover more skin while working may be protective [11]. Again, these factors have not been thoroughly investigated in South India. The role that nutritional status may play, another potential link to how poverty is associated with this disease, has also not been explored.

Method

This cross-sectional population study was performed in adults (≥18 years of age) in the Vellore District of Tamil Nadu in South India. A clustered sample design was used when recruiting participants. A list of villages and towns in the district was obtained from an online government database [17, 18]. These locations were mapped, and a smaller list of towns and villages within 30 km of the city of Vellore was generated. These 312 villages and 33 towns were then placed in lists with a single numeric value associated with each location, and 20 villages and 10 towns were randomly selected by means of a random number generator.

Attempts were made to recruit 21 participants from each village and 28 participants from each town to proportionately represent the approximately 60% rural population of Tamil Nadu [19]. Efforts were made to avoid recruiting more than one individual from a household. Patients were excluded if they were currently febrile, pregnant, had any immunosuppressing conditions, such as HIV or haematologic cancers, or were on immunosuppressing medications.

People who agreed to participate went through an informed consent process and then underwent a blood draw. Blood samples were stored on ice until they could be returned to the hospital, where they were stored at −70 °C until testing could be run. Prior infection with scrub typhus was detected by use of two enzyme-linked immunosorbent assays (ELISA), one to detect IgG and the other to detect IgM antibodies to scrub typhus. Both were processed using Scrub Typhus Detect (InBios International, Inc., Seattle, WA, USA) as per the manufacturer's specifications. This ELISA uses Karp, Kato, Gilliam and TA716 recombinant proteins of the 56-kD outer membrane protein and has a sensitivity of 84% and specificity of 98% for the IgM assay [20]. In the absence of consensus on what constitutes a 'positive' ELISA test in an endemic population, IgM cut-off values for optical density (OD) were set conservatively at >0.8. IgG cut-off values were set at>1.8. These values were obtained as the mean of the mixture distributions of IgM and IgG, respectively (Figures 1 and 2, respectively). The percentage of the participants with a positive serology –IgG, IgM or both – was taken as the seroprevalence for the Vellore area.

A risk factor survey was also administered to participants. This survey was translated into the local language, Tamil. Participants were asked the questions by personnel coached in administering the survey by the researchers. When possible, research personnel directly observed conditions, such as the proximity of the questioned items to a participant home or the proximity of bodies of water to a community. However, when this was not feasible, self-report was accepted instead. Questions were closed-ended, and answers were recorded on a predetermined form.

Biometric data – height, weight and mid-upper arm circumference – were also recorded on the same predetermined form, after being collected by personnel coached in taking these measurements.

Those who were subsequently found to have positive serologies, after the surveys were complete, were used as the case group, with those with negative serologies serving as the controls. Continuous data are summarised as means with standard deviations (SD). Categorical data are presented as frequencies and percentages. Each study variable was compared between cases and controls using conditional logistic regression. Further multivariable conditional logistic regression was performed including all variables with $P < 0.10$ in the univariate analyses. We examined the relationships between all explanatory variables and removed from the multivariable analysis variables which were highly correlated. Results are presented as odds ratios (ORs) and 95% CIs. All statistical analyses were conducted with Stata 11 (StataCorp, College

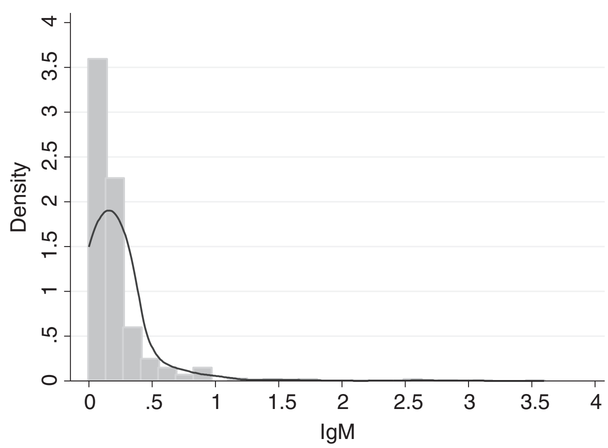

Figure 1 Distribution of IgM optical densities.

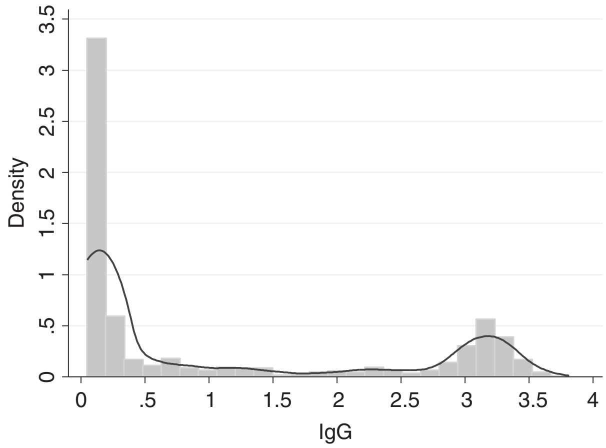

Figure 2 Distribution of IgG optical densities.

Station, TX, USA). This project was reviewed and approved by the Institutional Review Board and Ethics Committee of Christian Medical College in Vellore, Tamil Nadu.

Results

A total of 721 participants (62.6% female and 37.4% male) had serologies drawn and completed the risk factor survey. This included at least 28 participants from each urban area and at least 21 participants from each rural area, with the exception of one rural area where two blood samples were lost. The mean age of the participants was 50.6 years, ranging from 18 to 86 years of age. Rural participants made up 60.6% of those included in the study.

Overall, 229 of the 721 participants (31.8%) were found to be seropositive for scrub typhus. Seropositivity by IgG was found in 29.7%, with 4.2% being positive by IgM. Positivity by both IgG and IgM was found in 2.1% of the participants. Please see Table 1 for full details.

For analysis of risk factors, the 229 seropositive participants were used as cases, with the remaining 492 seronegative participants acting as controls. Grossly, the percentage of rural participants seropositive for scrub typhus was higher than that in urban areas (37.3% *vs.* 22.2%). However, this variable was not included in the analysis, as it was constant within each cluster. Risk factors significantly ($P < 0.05$) related to seropositivity by univariate analysis were female sex, older age, shorter height, lower weight, lower body mass index, smaller mid-upper arm circumference, fewer number of people in a household, fewer rooms in a house, having a house that was in a cluster with other houses and defecating in the open instead of using a toilet (Table 2).

Table 1 Patient Characteristics

Total Number of Participants	721
Mean Age	50.6 years
Sex	Female: 451 (62.6%)
	Male: 270 (37.4%)
Location	Rural: 437 (60.6%)
	Urban: 284 (39.4%)
Seropositivity	Positive: 229 (31.8%)
	IgG: 214 (29.7%)
	IgM: 30 (4.2%)
	Both: 15 (2.1%)
	Negative: 492 (68.2%)

The statistically significant variables from the univariate analyses were fed into a multivariate regression model. Height, weight and mid-upper arm circumference were significantly collinear with body mass index and were not used in the multivariate analysis. The number of rooms in a house and the number of people in a house were not, however, found to be significantly associated and were used. The variables which remained statistically significantly associated with seropositivity after multivariate regression modelling were age >60 years (OR= 3.50, P = 0.02), female sex (OR = 1.80, P = 0.02) and living in a house clustered near other homes (OR = 2.47, P = 0.02). As seen in the univariate analysis, there was a non-statistically significant trend towards greater seropositivity with older age in all categories past 30 years of age (Table 3).

Discussion

This study has several significant findings. First and foremost, the prevalence of scrub typhus in South India has risen dramatically over the last decade. Like many endemic areas, South India had virtually no reported scrub typhus for several decades, with only 5% prevalence after the disease was recognised to have re-emerged the early 2000s [9]. Since then, the prevalence has increased sixfold with nearly one-third of the local population now having evidence of past infection. Notably, our study used more conservative ELISA cut-off values than the prior study in South India. Additionally, the ELISA assay used in this study, although highly specific (98%), does have a lower sensitivity (82–84%) [20, 21]. Therefore, it is possible that our study understates the current prevalence. The recombinant 56-kD antigen used in this assay, based on Karp, Kato, Gilliam and TA716 strains, corresponds well with the Kato, Karp and Gilliam strains previously identified as the majority strains in this area of South India [22]. This certainly indicates that there is likely much mild or asymptomatic disease, as implied by

Table 2 Univariate analysis

Determinant	Category	Seropositivity	Odds Ratio (95% CI)	Significance
Animals	Animal (*n* = 211)	68 (32.2%)	1.06 (0.74–1.51)	0.75
	No Animals (*n* = 467)	145 (31.0%)	R	
Occupation	Agriculture/Laborer (*n* = 270)	93 (34.4%)	1.67 (0.74–3.77)	0.21
	Housewife (107)	22 (20.6%)	1.37 (0.49–3.84)	0.56
	Not Working (*n* = 232)	85 (36.6%)	1.73 (0.71–4.21)	0.23
	Office/Shop/Other (*n* = 102)	24 (23.5%)	R	
Agriculture	Rice/Vegetables (*n* = 46)	14 (30.4%)	0.88 (0.39–1.97)	0.78
	Other (*n* = 36)	6 (16.7%)	R	
House Type	Pucca/Tiled (*n* = 690)	220 (31.9%)	1.55 (0.65–3.69)	0.32
	Kutcha (*n* = 29)	9 (31.0%)	R	
Number of Rooms	(Per Room Increase)		0.84 (0.71–0.99)	**0.05**
Number of People	(Per Person Increase)		0.88 (0.79–0.98)	**0.02**
People/Room	(Per Person/Room Increase)		0.87 (0.69–1.08)	0.21
Bushes	Yes (*n* = 366)	107 (29.2%)	0.83 (0.63–1.10)	0.19
	No (*n* = 355)	122 (34.4%)	R	
Woodpile	Yes (*n* = 398)	129 (32.4%)	0.99 (0.65–1.50)	0.96
	No (*n* = 321)	98 (30.5%)	R	
Ditch	Yes (*n* = 292)	96 (32.9%)	1.30 (0.78–2.17)	0.32
	No (*n* = 427)	132 (30.9%)	R	
Water – Home	Yes (*n* = 145)	48 (33.1%)	0.60 (0.01–28.52)	0.80
	No (*n* = 528)	173 (32.8%)	R	
Water – Area	Yes (*n* = 387)	132 (34.1%)	1.86 (0.39–8.93)	0.44
	No (*n* = 334)	97 (29.0%)	R	
Home Cluster	Cluster (*n* = 663)	218 (32.9%)	2.12 (1.05–4.27)	**0.04**
	Individual (*n* = 53)	10 (18.9%)	R	
Work Top*	Shirt (*n* = 199)	58 (29.1%)	1.25 (0.20–7.66)	0.81
	Banian (*n* = 16)	4 (25.0%)	R	
	None (*n* = 1)	1 (100%)	Excluded	
Work Bottom*	Pants (*n* = 55)	13 (23.6%)	0.69 (0.29–1.66)	0.40
	Lungi (*n* = 155)	49 (31.6%)	R	
	Shorts (*n* = 5)	0 (0.0%)	Excluded	
Home Top*	Shirt (*n* = 199)	59 (29.7%)	2.89 (0.65–12.90)	0.16
	Banian (*n* = 13)	1 (7.7%)	R	
	None (*n* = 2)	1 (50.0%)	Excluded	
Home Bottom*	Pants (*n* = 47)	11 (23.4%)	0.51 (0.19–1.35)	0.18
	Lungy (*n* = 162)	50 (30.9%)	R	
	Shorts (*n* = 5)	0 (0.0%)	Excluded	
Bathing	Whole Body (*n* = 531)	169 (31.8%)	0.60 (0.12–2.94)	0.53
	Hands/Arms (*n* = 145)	46 (31.7%)	0.90 (0.18–4.45)	0.90
	None (*n* = 26)	11 (42.3%)	R	
Defecation	Field/Bushes (*n* = 293)	94 (32.1%)	1.48 (1.01–2.18)	**0.04**
	Toilet (*n* = 409)	131 (32.0%)	R	
Sex	Female (*n* = 451)	147 (32.6%)	1.60 (1.05–2.45)	**0.03**
	Male (*n* = 270)	82 (30.4%)	R	
Age	<20 (*n* = 7)	0 (0.0%)	0 (0)	0
	30–40 (*n* = 140)	33 (23.6%)	1.17 (0.46–2.98)	0.73
	40–50 (*n* = 169)	47 (27.8%)	1.74 (0.72–4.27)	0.22
	50–60 (*n* = 141)	49 (34.8%)	2.31 (0.82–6.51)	0.11
	>60 (*n* = 197)	86 (43.7%)	3.06 (1.22–7.68)	**0.02**
	20–30 (*n* = 67)	14 (20.9%)	R	
Height	(Per cm Increase)		0.96 (0.93–0.98)	**0.001**
Weight	(Per kg Increase)		0.97 (0.96–0.98)	**<0.001**
BMI	(Per kg/m^2 Increase)		0.97 (0.94–0.99)	**0.02**
MUAC†	(Per cm Increase)		0.94 (0.90–0.98)	**0.01**

*Measured in men only.
†Mid-upper Arm Circumference.
Significant *P*-values (<0.05) are marked in bold.

Table 3 Multivariate analysis

Determinant	Category	Odds ratios (95% CI)	Significance
Number of Rooms	(Per Room Increase)	0.95 (0.79–1.16)	0.66
Number of People	(Per Person Increase)	0.94 (0.84–1.05)	0.25
Home Cluster	Clustered	2.47 (1.20–5.12)	**0.02**
	Individual	R	
Defecation	Field/Bushes	1.23 (0.75–2.02)	0.42
	Toilet	R	
Sex	Female	1.80 (1.10–2.92)	**0.02**
	Male		
Age (years)	30–40	1.09 (0.38–3.18)	0.87
	40–50	2.08 (0.76–5.73)	0.16
	50–60	2.52 (0.85–7.44)	0.09
	>60	3.50 (1.32–9.29)	**0.02**
	20–30	R	
BMI	(Per kg/m^2 Increase)	0.97 (0.94–1.00)	0.09

Significant *P*-values (<0.05) are marked in bold.

Table 4 Physical and nutritional risk factors for scrub typhus

Determinant	Positive†	Negative†
Age (years)	55.0 (14.6)	48.6 (14.8)
Height (cm)	153.7 (8.9)	155.3 (9.0)
Weight (kg)	59.9 (12.8)	62.1 (13.9)
BMI (kg/m^2)	25.4 (5.1)	25.8 (5.4)
MUAC (cm)*	26.2 (3.4)	26.5 (3.4)

*Mid-upper Arm Circumference.
†Mean (SD).

the relatively low mortality rates sound in some studies [1]. However, as it is unclear why some people develop severe disease and others do not, thus prevention remains a primary concern.

Although the stereotyped pattern of this being a rural disease held true in our study, none of the previously described risk factors regarding the proximity of a person's home to mite-friendly surroundings were associated with infection in our study. This may be because Vellore is situated within large tracts of scrubland. Individual risk factors may matter less when the nature of the surrounding land is so conducive to bringing humans into contact with mites.

Although the proximity of mite-friendly surroundings to an individual home does not seem to be a risk factor in South India, the proximity of other homes is. Somewhat surprisingly, people who lived in homes that were closely clustered with other homes were more likely to be seropositive. It might have been anticipated that people living in individual houses surrounded by wild land would more likely to be seropositive. This was not the case. A potential explanation for this could have been that the disease is associated with crowding or factors such as the role of rodents as amplifiers of infection to humans [14, 15]. However, the lack of association between the number of people per room in a person's home with disease exposure speaks against this. It seems likely that home clustering serves as a proxy for a confounding variable, such as socio-economic status, but this would have to be further explored.

As with the less modifiable factors such as housing location, so too there were few associations found between personal habits and scrub typhus exposure in South India. Notably, as was previously found in North India [16], toileting outdoors was significantly associated with disease exposure on univariate analysis. With multivariate modelling, however, this association disappeared. Toileting in vegetation may still be associated with scrub typhus. The vast majority of those in our study who did not use toilets reported defecating in fields, and the single individual who reported defecated in the bushes had been exposed to scrub typhus before. The prior study also specifically looked at squatting to toilet [16], which we did not specify. Bathing habits after work and the type of clothing worn by men had no significant association with disease exposure in our study, although they had in prior studies. It is worth noting, however, that there were very small numbers of participants in several categories, and the single individual in our study who worked without a shirt was seropositive. Like personal habits, occupation was not significantly associated with scrub typhus exposure in South India.

Although, as might be anticipated, there was a trend towards those working outdoors (farmers and labourers) being more likely to be seropositive than those working indoors (office workers), there were not any statistically significant differences in exposure to scrub typhus among people of different occupations. The reason for this is unclear. It could be, once again, that if the nature of the environment in the Vellore area is conducive to scrub typhus exposure, the individual risk factors have less of an impact. If the location of an individual's residence does not significantly change the risk of being exposed to scrub typhus, why would the location of their work, where they are likely to spend less of their time? Alternatively, this lack of significant difference among occupations may represent an artefact from our study. Not only did many individuals have more than one occupation, which may have confounded our findings, but we also recruited participants primarily within villages and towns, where people were more readily accessible, rather than from the surrounding countryside. The prevalence of scrub typhus among the farmers and labourers recruited from these populations centres may not be representative of that found among those living further out. No association was found between the type of agriculture and seropositivity in our study, although our numbers were quite small in these categories. The high level of scrub typhus exposure among those not working is somewhat unexpected as well. Certainly, many in this group were older and retired, and scrub typhus exposure was strongly associated with older age.

The strong association of scrub typhus exposure with older age and female sex is somewhat surprising. The association with female sex has been previously reported in Malaysia [14], Korea, Thailand and Japan [23]. The reason for this association has been speculated to be the differing roles of men and women in those societies, with rural women spending more time doing the types of work associated with scrub typhus acquisition. Alternatively, the association with female sex may represent a selection bias within our study. Men were very difficult to recruit. The higher seroprevalence of scrub typhus among women may represent an under-representation among men, as only men not working or sleeping during the day were in the population centres to be recruited for our study. The reason for the strong association between prior disease exposure and older age also remains unclear. Accumulated life exposure may play a role in this, as those currently past the age of 60 would have been in the prime of their working lives when scrub typhus began re-emerging 20 to 30 years ago. It is less likely that this represents continued seropositivity from before the disappearance of scrub typhus much earlier, as the antibody response is thought to fade fairly rapidly. Our own prior unpublished results concur with prior literature [24], showing that IgG titres to scrub typhus falls rapidly below the limits of detection, generally in less than 36 months. It must also be considered, then, that scrub typhus was more common than previously believed and that it was undiagnosed by clinicians and under-represented in the prior study or that there are other unexplored factors that make the elderly more susceptible to infection.

Finally, the role of nutritional status in the acquisition of scrub typhus remains unclear. With every one of the tested parameters being associated with scrub typhus exposure, it seems unlikely that there is no connection between scrub typhus infection and nutritional status. However, the absolute differences between cases and controls were very small (Table 4). It is possible that undernutrition predisposes one to acquiring scrub typhus. Alternatively, it seems likely that chronic malnourishment and a higher likelihood of being exposed to scrub typhus may both be consequences of a confounding variable such as poverty or baseline poor health. Further research in this area would need to be performed to ascertain the nature of this relationship.

Certainly, there are limitations to this study. A clustered design was used for convenience, as a randomised representative sampling of the entire Vellore district would have been prohibitively large and expensive. By restricting our sampling to within 30 km of Vellore, the generalisability may be less. It was also difficult to recruit working individuals particularly young men. Ideally, a larger representative survey of a broader area of South India that worked to motivate young men to participate would be performed to confirm our findings. Finally, the assay used to assess prior exposure may be insensitive, particularly as prior studies looking at the sensitivity were carried out in symptomatic individuals and still only found an 82–84% sensitivity [20, 21]. We used more stringent cut-offs, which, although increasing the specificity likely decreased the sensitivity further. However, the natural consequence of this potential weakness in the chosen assay would be to moderate rather to exaggerate our findings, further underscoring the dramatic rise in the prevalence of scrub typhus in South India.

Conclusions

Overall, it is quite clear that scrub typhus has become a common infection in South India over the last decade. Unfortunately, our study has failed to turn up significant targets for public health educational interventions, as none of the associated risk factors that we have found are readily modifiable by individuals. Why women are more likely to be exposed to scrub typhus, as noted in prior studies, remains to be explained. Further evaluation into how differences in gender roles may play in the likelihood of scrub typhus exposure is warranted.

Regardless, several things must happen. First and foremost, clinicians in South India must be made aware of the extent of this problem. Many physicians remain ignorant that this disease even exists, much less its extent. Even when physicians are aware, diagnostics remain inadequate, and further research is warranted in this area. There are many promising signs for using polymerase chain reaction (PCR) technology in this regard, although this has not yet truly come to fruition. Ultimately, barring eradication or severe curtailing of the vector that carries this disease, vaccination remains the most promising approach. Vaccines against scrub typhus have been attempted in the past by the US military during the Vietnam War. Unfortunately, they met with little success and lost support after the end of armed conflict in the region. However, vaccine efforts have renewed in several scrub typhus endemic regions. It is vital to public health efforts that these endeavours are supported, as this may ultimately end up being the most realistic way to control this dangerous and increasingly common disease.

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