

# VIRAL RESPIRATORY TRACT INFECTIONS AMONG PATIENTS WITH ACUTE UNDIFFERENTIATED FEVER IN VIETNAM

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**Abstract.** To investigate the proportion of viral respiratory tract infections among acute undifferentiated fevers (AUFs) at primary health facilities in southern Vietnam during 2001-2005, patients with AUF not caused by malaria were enrolled at twelve primary health facilities and a clinic for malaria control program. Serum was collected on first presentation (t0) and after 3 weeks (t3) for serology. After exclusion of acute dengue infection, acute and convalescent serum samples from 606 patients were using enzyme-linked immunoassays to detect IgA, as well as IgM and IgG antibodies against common respiratory viruses. Paired sera showed the following infections: human parainfluenza virus (HPIV, 4.7%), influenza B virus (FLUBV, 2.2%), influenza A virus (FLUAV, 1.9%) and human respiratory syncytial virus (HRSV, 0.6%). There was no association between type of infection and age, sex or seasonality; some inter-annual differences were observed for influenza. Antibody prevalence, indicative of previous infections, was relatively low: HPV, 56.8%, FLUBV, 12.1%; FLUAV, 5.9% and HRSV, 6.8%.

**Key words:** viral respiratory tract infection, acute undifferentiated fever, Vietnam

## INTRODUCTION

Data regarding the etiology of acute respiratory tract infections (ARIs) in developing tropical countries are sparse. The available data are based on sero-surveys or on diagnostic studies in defined cases of respiratory tract infection (Weber *et al*, 1998;

Puzelli *et al*, 2006). Little is known about respiratory tract infections among patients with fever who present to primary health facilities in developing countries. ARIs contribute significantly to mortality, especially among children in developing countries (WHO, 1998). Global threats, including the influenza pandemic, or potentially fatal disease, such as avian influenza and severe acquired respiratory syndrome (SARS), can be hidden among ARIs. It is therefore important to know more about the epidemiology, presentation and options for early detection of these infections.

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Viruses, such as human adenovirus (HAdV), human respiratory syncytial virus (HRSV), influenza A virus (FLUAV), influenza B virus (FLUBV), human parainfluenza virus (HPIV), human enteroviruses and human rhinoviruses, are important causes of ARI worldwide (Tumova *et al*, 1989; Shek and Lee, 2003; Bourgeois *et al*, 2006). ARIs affect mostly children and the incidence decreases with increasing age (Yun *et al*, 1995; Tsai *et al*, 2001; Shek and Lee, 2003). As respiratory viruses are highly contagious and easily transmitted by aerosols or direct contact, they may cause epidemics. SARS (caused by SARS-associated human coronavirus) is an example of a new virus that soon after its emergence threatened to evolve into a pandemic with a high case fatality rate (Peiris *et al*, 2003). In contrast, avian influenza (influenza A virus H5N1), is an example of a virus that is highly pathogenic to humans but not very infectious (Hayden, 2006; Thomas and Noppenberger, 2007). It has a large volatile bird reservoir which precludes control and causes repeated outbreaks in the poultry industry worldwide.

In Vietnam the incidence of communicable diseases has fallen in recent decades, but respiratory tract infections still rank high among the ten leading causes of morbidity (WHO-WPRO, 2004, 2005). The morbidity data is mainly based on routine reporting by health services which may not reflect the total burden of disease, similar to what is seen with dengue fever (Phuong *et al*, 2006c). There are no good estimates of the incidence of respiratory tract infections in Vietnam. In 1980 an ARI control program was established, which became a national program in 1984, focusing on children (WHO, 1992). This program was set up with the goal to diagnose and treat 80% of the nationwide occurring ARIs by 2000. In 1998, despite high cover-

age (87.5%) by trained health workers and drug availability, it was estimated that only 20% of children with ARI were being treated according to guidelines (UN, 1999). For older children, adolescents and adults there is no data.

The surveillance of ARI does not include confirmation by laboratory techniques. If viral or bacterial cultures, PCR or serological tests are performed, this is usually done in hospitals or medical centers for individual case management rather than for routine surveillance on a broader scale. Uncomplicated cases who present to primary health facilities usually do not receive a classifying diagnosis (Phuong *et al*, 2006a).

A discrepancy between low detection rates for ARIs and high morbidity associated with ARI is possibly due to underreporting of early, uncomplicated, and clinically non-specific stages. In this study we investigated how many ARIs are found within the group of undifferentiated fevers for which patients seek help at community primary health centers and report the sero-prevalence of ARIs in southern Vietnam.

## MATERIALS AND METHODS

### Study sites and population

The study enrolled patients with acute undifferentiated fever who sought help at one of twelve non-adjacent community health centers (here after called health posts), and one clinic at the provincial malaria station, in Binh Thuan Province. Details of the study sites were described previously (Phuong *et al*, 2006a). Binh Thuan Province has a population of approximately 1.2 million living in an area of 7,992 km<sup>2</sup>. The tropical monsoon climate causes an annual average temperature of 26°C with a hot and dry season alternating

with a rainy season that lasts from May to October. The annual rainfall averages 1,400 mm. Agro-forestry, fishing and recently tourism are the major sources of income.

The study started in March 2001. Patients with acute undifferentiated fever (AUF), who presented to the study health posts, were asked to participate in the study. AUF was defined as any febrile illness of less than 14 days duration, confirmed by an axillary temperature  $\geq 38.0^{\circ}\text{C}$ , without evidence of severe systemic or organ specific disease. Pneumonia, documented by percussion and or auscultation of crackles or rales, was an exclusion criterion. X-ray facilities were not available. Malaria was excluded by microscopic examination of a thick blood smear. Study case record forms were filled in by the attending healthcare workers, including identifiers (age, sex, occupation, and address), recent exposure factors (contact with fresh water or flooded terrains, work in paddy fields, visit to a forest or work in a forest), characteristics of disease (duration, symptoms and signs, including the results of the tourniquet test), self-treatment, a presumptive diagnosis, prescribed treatment, referral and final outcome. Presumptive diagnoses, such as "acute fever" and "viral infection", were reclassified as "undifferentiated fever". Two blood samples were collected by venous puncture on presentation, at time 0 (t0), and 3 weeks later (t3). Sera were stored at  $-20^{\circ}\text{C}$  at the study sites until monthly transfer to Cho Ray Hospital, where they were stored at  $-70^{\circ}\text{C}$ .

Serological testing for respiratory virus infections was only done for patients in whom paired serum samples had been collected and only after dengue had been excluded as a cause of the fever. March 2001 through March 2002, patients who complained of mild to moderate cough

were selected in order to get a first impression. The t3 sera of 126 patients were screened for antibodies to influenza A virus (FLUAV), influenza B virus (FLUBV), human respiratory syncytial virus (HRSV) and human adenovirus (HAdV). Later, the sera of 22 patients who did not complain of cough, were also examined.

During the following years, patients were selected randomly, (two per month per health post) and those who tested negative were further tested for respiratory viruses. Cough was not a selection criterion any more. During the second year, from April 2002 through March 2003, and the third year, from April 2003 through March 2004, t3 sera from 138 patients (equally divided over periods 2 and 3) were screened for FLUAV and HRSV. During the final year, January 1 to December 31, 2005, paired serum samples from 320 patients were tested for FLUAV, FLUBV, HRSV and Human parainfluenza virus 1, 2, 3 (HPIV-1; HPIV-2; HPIV-3).

### Serology

During Year 1 a monowell combined capture ELISA was used for the simultaneous detection of IgM and IgA antibodies to FLUAV, FLUBV, HRSV, and to HAdV (Meddens Diagnostics BV, Vorden, the Netherlands). The tests were performed according to the instructions of the manufacturer on convalescent (t3) samples at the laboratory of the Department of Virology, Erasmus MC, Rotterdam, the Netherlands.

During Years 2, 3 and 4, ELISA panels from another manufacturer were used (Virion\Serion GmbH, Würzburg, Germany). Separate panels were used to detect IgG and IgA antibodies to FLUAV, FLUBV, and HRSV. For HPIV an IgA ELISA assay was used that was developed to diagnose acute infection but does not

discriminate between the three HPIVs. For IgG separate panels were used for HPIV-1 and HPIV-2. The tests were performed according to the instructions of the manufacturer. For Years 2 and 3 only the t3 samples were tested in the laboratory of virology of Cho Ray Hospital; for Year 4 both t0 and t3 samples were tested at the laboratory of the Department of Virology, Erasmus MC, Rotterdam, the Netherlands.

### Interpretation of the serological results

During the first three years the diagnosis was based on the t3 sample results only, based on the concept that the detection of IgA, IgM or IgG antibodies in the convalescent sample was indicative of an acute infection (Meddens *et al*, 1990; Voeten *et al*, 1998; Rothbarth *et al*, 1999). During the first year a positive combined IgM/IgA test kit of Meddens Diagnostics was interpreted as indicative of "acute infection" (Varsano *et al*, 1995). With the Serion ELISA assays, used in the later periods, a positive IgA and/or IgG was classified as acute/past infection. Later, it was acknowledged, for HPIV and HRSV, (recent) past infections could not be clearly discriminated from acute infections on the basis of a single test result only. Therefore during the last year the tests were expanded to testing both the acute and convalescence samples. These results were classified as acute infection, past infection and no infection. Acute infection was defined as a positive test on the t3 sample and a fourfold increase in IgA concentration and/or IgG between t0 and t3. A positive IgA or IgG tests at t3 without seroconversion was classified as a past infection. No evidence of infection was diagnosed when neither IgA nor IgG was detectable at t3.

### Ethical considerations

Medical ethical clearance for the study was obtained from the Scientific Committees of Cho Ray Hospital in Ho Chi Minh

City and the Binh Thuan Provincial Health Service. The study was explained and discussed in meetings with provincial authorities and the staff of the health posts. All patients, (or in children, the parents or guardian) gave written informed consent prior to participation.

### Data analysis

All data were stored in Microsoft Access where patient selection and randomization was done. SPSS for Windows (version 14.0; SPSS, Chicago, IL) was used for statistical analysis. Descriptive statistics were calculated for background variables and a chi-square test was used to compare categorical factors.

## RESULTS

In total, 606 patients were included in the study. The general characteristics of the study population are shown in Table 1. Overall there was no difference in respect to age, sex, occupation and season between patients with "cough" and without "cough". In 2005 ARIs were more common among those who reported cough (chi-square 10.625, *df* = 1, *p*-value = 0.001) suggesting the initial selection of patients in the 2001 cohort had more ARI.

In 2001 and 2005, approximately 8% of t3 samples tested positive for HRSV and 1% of test results showed acute infection, whereas in 2002 and 2003 none of the tested samples were positive for IgA or IgG. For influenza we observed a great inter-annual difference. Six percent of t3 samples in 2005 were positive for FLUAV, whereas in previous years this was 18% or higher. In 2005, approximately 11% of samples tested positive for FLUBV, whereas in 2001 25% of samples tested positive for FLUAV and FLUBV. The results of serological screening in Years 1 to 3, and the expanded test results in Year 4 based on the single t3

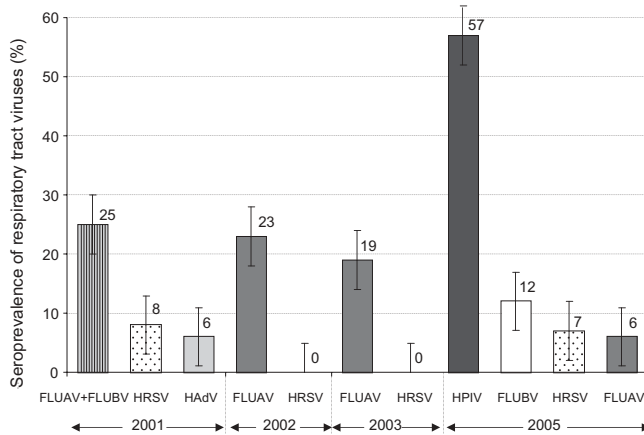


Fig 1–Seroprevalence of respiratory tract viruses from 2001 to 2005 in Binh Thuan Province, southern Vietnam.

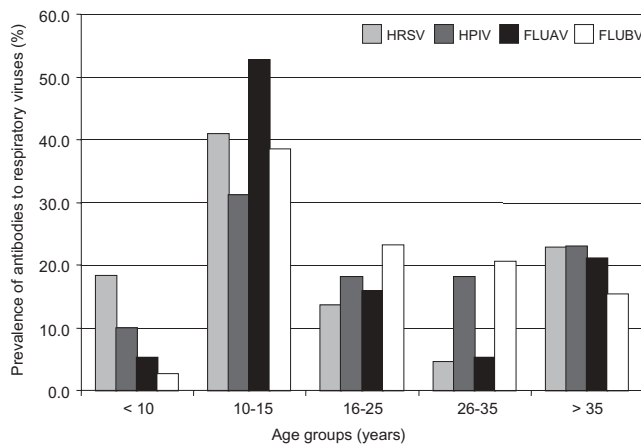


Fig 2–Prevalence of antibodies to respiratory tract viruses by age in Binh Thuan, southern Vietnam.

test results, are shown in Table 2. The seroprevalence of respiratory tract viruses from 2001 to 2005 in Binh Thuan Province, Vietnam is shown in Fig 1.

In 2005 acute viral respiratory infections caused 10.0% (32) of AUFs. The contributions by HPIV, FLUBV, FLUAV and HRSV were 4.7, 2.2, 1.9 and 0.6%, respectively. The diagnosis of HPIV was based on seroconversion of IgA in 5 cases, IgG in 10 cases and both IgA and IgG in 0 cases.

FLUAV was diagnosed by 4, 0, 2, for FLUBV by 2, 3, 2 and for HRSV infection by 1, 1, 0 by seroconversion of IgA, IgG and both IgA and IgG, respectively. Mixed antibody responses were found in two cases (0.6%). One was a 12 year-old girl with a greater than fourfold increase in FLUAV-IgG (9.8 times), FLUBV-IgG (19.4 times), HRSV-IgG (4.7 times) and HPIV-2-IgG (4.3 times); the other patient was a 39 year-old farmer with a 3.9 fold increase in FLUAV-IgA and a 3.8 fold increase in HPIV-IgA. In 124 patients (38.5%) no antibodies were found in any of the tests.

#### Characteristics of acute respiratory viral infections

For further analysis of the data, only the results from 2005 were used. The characteristics of the 32 patients with serologically confirmed acute viral respiratory tract infection were further analyzed. There were no significant differences in age or seasonality of the virus infections. There were no significant differences between the symptoms and signs of patients with a serodiagnosis of acute viral respiratory infection and those who had negative results.

The responses of the primary health care workers were uniform. A presumptive diagnosis of acute fever was diagnosed in 290 patients (47.9%) (37.5% vs 48.4% in those with and without acute respiratory infection, respectively); pharyngitis was diagnosed in 214 patients (35.3%) (43.8% vs 34.8%); dengue fever was diag-

Table 1

General characteristics of patients presenting with acute undifferentiated fever at primary health posts in Binh Thuan Province, Vietnam who were serologically tested for respiratory tract viruses.

	Year			
	2001	2002	2003	2005
Total number	148	69	69	320
Median age (range)	27.0 (4 - 74)	28.3 (7 - 75)	21.3 (4 - 76)	16.8 (5 - 74)
Age groups, no. (%)				
<10 yrs	11 (7.4)	5 (7.2)	8 (11.6)	40 (12.5)
10-15 yrs	26 (17.6)	5 (7.2)	19 (27.5)	105 (32.8)
16-25 yrs	34 (23.0)	21 (30.4)	13 (18.8)	63 (19.7)
26-35 yrs	44 (29.7)	17 (24.6)	9 (13.0)	58 (18.1)
>35 yrs	33 (22.3)	21 (30.4)	20 (29.0)	54 (16.9)
Gender F/M, no.	61/87	27/42	37/32	123/197
Season, no. (%)				
Rainy season	99 (66.9)	34 (49.3)	39 (56.5)	163 (50.9)
Dry season	49 (33.1)	35 (50.7)	30 (43.5)	157 (49.1)
Occupation, no. (%) <sup>a</sup>				
Farmer	85 (57.4)	42 (60.9)	35 (50.7)	110 (34.4)
Pupil	33 (22.3)	15 (21.7)	24 (34.8)	157 (49.1)
Others	20 (13.5)	09 (15.9)	10 (14.5)	50 (15.6)

<sup>a</sup>Missing 10 in 2001, 3 in 2002 and 3 in 2005

nosed in 55 patients (9.1%) (15.6 % vs 8.7%) with a *p*-value of 0.265. For treatment 61.3% of patients received at least one antibiotic (196/320); this was similar for patients with acute viral respiratory infection and patients without infection (59.4% and 61.5%, respectively, *p*=0.818). Complete recovery was recorded in 301 patients (94.1%) at t3 and partial recovery in 14 patients (4.4%). In 5 patients (1.6%) this information was missing.

The monthly distribution of acute viral infections was different from health post to health post, but in general it showed a scattered pattern in which no seasonality could be discerned. In 4 health posts no infections were detected throughout the year. The prevalence of IgG anti-

bodies indicated no community was free of these respiratory tract infections.

### Seroprevalence of respiratory viruses

The seroprevalence of respiratory viral infections was estimated based on the presence of either IgA or IgG antibodies at t3. For HPIV this was 56.8%, FLUBV 12.1%; FLUAV 5.9% and HRSV 6.8%. The participants were classified into 5 age groups: <10 years, 10-15, 16-25, 26-35, and > 35. The distribution of respiratory viruses by age group is shown in Fig 2. There was no differences between males and females.

### DISCUSSION

This study shows the common etiologic agents of respiratory tract infections

Table 2

Serological indications of acute respiratory tract infection in patients with undifferentiated fever at primary health posts in Binh Thuan Province, Vietnam.

Tested serum samples		No. patients (%) with positive antibodies				
		Convalescence			Paired	
		2001	2002	2003	2005	
Year of study						
Number of patients tested		<i>n</i> = 126 <sup>a</sup>	<i>n</i> = 22 <sup>b</sup>	<i>n</i> = 69 <sup>b</sup>	<i>n</i> = 69 <sup>b</sup>	<i>n</i> = 320 <sup>b</sup>
HAdV	Acute	} 7 (6)				
	Past					
FLUAV or FLUBV	Acute	} 31 (25)				
	Past					
FLUAV	Acute	} 4 (18)	} 16 (23)	} 13 (19)	8 (3) <sup>c</sup>	
	Past				11 (3)	
FLUBV	Acute				8 (3) <sup>c</sup>	
	Past				30 (9)	
HPIV- (1+2+3)	Acute				17 (5) <sup>c</sup>	
	Past				166 (52)	
HRSV	Acute	} 10 (8)	} 0	} 0	} 0	3 (1) <sup>c</sup>
	Past					19 (6)

HAdV, Human adenovirus; FLUAV, Influenza A virus; FLUBV, Influenza B virus; HPIV, Human parainfluenza virus; HRSV, Human respiratory syncytial virus

<sup>a</sup>Tested with a combined IgA and IgM ELISA; <sup>b</sup>Tested with separate IgA and IgG ELISA.

<sup>c</sup>There were two mixed antibody responses: 1 case with antibodies against FLUAV, FLUBV, HPIV and HRSV and one case with antibodies against FLUAV and HPIV.

t0, serum sample taken upon presentation to the primary health facility;

t3, convalescent serum sample taken three weeks after t0

circulate in Binh Thuan Province but their contribution to undifferentiated fevers causing people to seek medical help is small. Together, they comprise approximately 10% of undifferentiated fever in patients older than four years. Human parainfluenza virus infection was the most common.

The study was health service based aimed at finding causes of undifferentiated fever in patients who sought help at public primary health facilities. It was not an epidemiologic surveillance of viral respiratory infections, and the results may not reflect the true epidemiology of viral respiratory infections in Binh Thuan Province.

The study only addressed the most common viral agents that cause respiratory tract infections with fever and did not investigate the less frequent causes of ARI in Vietnam. However, the results contribute to a better understanding of the epidemiology of viral respiratory tract infections. Another limitation of this study was that collecting blood from children younger than four years of age, was almost always refused. Since respiratory viruses circulate intensively among children under age five, this study probably underestimates the true incidence of acute respiratory tract infections.

The seroprevalence found in this

study is somewhat lower than what was found in other tropical regions (Weber *et al*, 1998; Yeolekar *et al*, 2001; Puzelli *et al*, 2006). The differences in seroprevalence may reflect differences in patient selection or differences in tests used, but the relatively low seroprevalence of IgG indicates the true incidence of these viral infections is relatively low in Binh Thuan.

Mixed antibody responses occurred in two cases in 2005. The diagnosis in these two cases is not clear, but the low rate of such non-specific, polyvalent responses indicates the assays used were specific enough to make a diagnosis in the majority of patients. By including the results of the t0 sample the specificity of the serodiagnosis in 2005 was further enhanced. The incidences of the respective respiratory tract infections in 2005 are conservative estimates.

IgG antibodies remain detectable for at least two to three years after FLUAV and FLUBV, and for one year after infections with HRSV and other paramyxoviruses (Couch and Kasel, 1983; Ogra, 2004). Viral respiratory tract infections are common throughout life and humans do not develop lifelong (cross protective) immunity to different virus types (Crowe and Williams, 2003). HRSV infections, that occur after the first two years of life, are almost always reinfections that tend to become progressively milder (Crowcroft *et al*, 1999; Hall and McCarthy, 2005). HPIV reinfections in immunocompetent individuals are more likely to cause mild upper respiratory tract infections, with sparing of the lower respiratory tract after the first or second exposure (Welliver *et al*, 1982; Glezen *et al*, 1984). Infection of influenza viruses yields longstanding immunity to reinfection with homologous viruses but offers essentially no protection against other (sub)types (Couch and

Kasell, 1983). This, antigenic drift and shift of influenza viruses, explain why influenza continues to cause major epidemics (Treanor and Falsey, 1999). During the study period avian influenza was introduced into Vietnam, but no avian influenza virus A (H5N1) infections were detected in Binh Thuan Province (MOH Vietnam, 2005; OIE, 2007).

It is difficult to distinguish between the different viral respiratory tract infections on clinical grounds only (Nicholson *et al*, 1997). This has been addressed in many studies focusing on children, but has only recently received more attention in adults (Dowell *et al*, 1996; Crowcroft *et al*, 1999; Treanor and Falsey, 1999; Hall, 2001; Mangtani *et al*, 2006). The manifestations of viral respiratory tract infections in adults are highly variable, ranging from asymptomatic infection to lower respiratory tract involvement and severe disease in high risk populations (Treanor and Falsey, 1999; Hall, 2001; Zambon *et al*, 2001; Yang *et al*, 2003).

Seasonality of the incidence of viral respiratory tract infections was not observed in this study. Documented seasonal patterns of viral respiratory infections in the tropics suggest HRSV and influenza virus infections are associated with rainfall but this was not confirmed in this study (Chew *et al*, 1998; Shek *et al*, 2003). The inter-annual differences in the rates of FLUAV and FLUBV infections in this study may be partly explained by differences in testing. In 2001 a combined FLUAV and FLUBV test was used and during the first years of the study only the t3 samples were tested, whereas in 2005 the criterion of increasing antibody concentrations was added. The number of FLUAV positive (IgA + IgG) t3 samples was still lower in 2005 than in the two previous years, and the number of FLUAV

and FLUBV positive samples was higher than in 2001. There is not much known about inter-annual variation in influenza incidence in Southeast Asia. In temperate zones this is a well known phenomenon and our results point to similar fluctuations in the tropics.

In this population the contribution of common viral respiratory infections to the causes of AUF was small. Respiratory viruses, especially influenza, may spread rapidly because it has a short generation time, even if it has low transmissibility or does not spread to many others (Carrat *et al*, 2008). Approximately one-fourth to one-third of AUFs can be attributed to dengue fever (Phuong *et al*, 2006b,c) while the cause of the remaining fevers is unknown. One serosurveillance indicated brucellosis was unlikely to be a cause of AUF (Nga *et al*, 2006). Leptospirosis is of interest since IgM and IgG antibodies are found (Wagenaar *et al*, 2004; Thai *et al*, 2006) but how much leptospirosis contributes to AUF still has to be evaluated. No evidence of histoplasmosis was found in surveillance of 180 selected samples from 2002 to 2005 and antibodies to Chikungunya virus and rickettsia species were rare (unpublished data). There are no epidemiologic data regarding infectious diseases in this province to direct further investigations. Less frequent air borne pathogens have not been studied extensively.

From an operational point of view it is good news the incidence of viral respiratory tract infections among these patients with undifferentiated fever was low and that none progressed to severe respiratory tract infections. This means there is little overlap between AUF and the case definitions that are applied in the ARI program, which are based on the clinical findings of respiratory tract infections. There is no need to expand the ARI program to

a syndromic approach for undifferentiated fever. The bad news was antibiotic prescription rates were inordinately high for infections that do not require (empiric) antibiotic therapy.

In conclusion, acute respiratory tract infections are an infrequent cause of undifferentiated fever in Binh Thuan. The transmission of respiratory viruses is relatively low in Binh Thuan. A study focusing on infections in children is necessary to complete the assessment of the impact of viral respiratory tract infections in the community.

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