

Analysis and Research on Distribution of Ticks and SFTSV and HGA Carried by Ticks in Prevalence Area in South of Henan

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Keywords: South of Henan, Ticks, Severe Fever with Thrombocytopenia Syndrome, Anaplasma Phagocytophilum, Pathogen

Abstract. Objective: To make a study of the distribution features of ticks in south of Henan and the SFTSV and HGA. Methods: From January to December in 2015 in Xinyang region of Henan Province the artificial cloth flag method and surface pick tick method were collected field of free ticks insect and animal body parasitic ticks, method for the determination of tick borne pathogens were analyzed by PCR and sequence, immunity was used to determine the serum of host pathogen. Results: ticks were collected by 1235, of which 473 are free ticks and 762 are parasitizing ticks. Fever tick specimens and host in the serum samples of thrombocytopenia to reduce the overall syndrome virus, phagocytophilum Anaplasma nucleic acid detection showed positive. Conclusion: The epidemic happening rate is related with the distribution of ticks and SFTSV and HGA carried by ticks. The fever with thrombocytopenia syndrome virus and the non-form of the phagocytic cells were detected in the serum of the tick host, which did not exclude the possibility of the transmission of the human.

Introduction

Ticks belong to the animal kingdom (Animal Kingdom), the arthropod door (Arthropoda), Arachnida (Arachnida), acari (acari), send Acarina (parasitiformes), menservants suborder ixodida), Ixodoidea (Ixodoidea), is terrestrial vertebrate obligate and non-permanent ectoparasites; since 2009, Xinyang City, Henan Province, often "fever with blood platelet decrease syndrome" and anaplasmosis reported that patients with life in the mountain area in agricultural production mainly, some patients have history of tick bite. In order to understand the Henan Province Xinyang tick species, seasonal fluctuation and carry new bunyavirus, addicted to phagocytic cells form (HGA). From time to time in the grass or bushes, lot, capturing free ticks using artificial cloth flag (100cm*100cra, white cloth made of); the persistent active surface against hair picking up method, in domestic animals such as cattle, sheep, dogs and ticks were collected. The collected ticks were identified by morphological classification under laboratory microscope, counting the density of different habitats in different months (only / the flag of artificial cloth per hour or only / every animal body). The collected ticks on the ice grinding after extracting nucleic acid was detected by PCR, the PCR products were purified and cloned and sequenced, the determination of nucleic acid sequence by DNASTar seqman were assisted proofreading, then homology comparison with GenBank published sequences were compared and analyzed using megalign software, nucleic acid sequence alignment analysis using DNASTar. By this time in Xinyang, Henan Province tick

populations, distribution, seasonal dynamics and new Bunia virus carrying research, master in Xinyang, Henan Province tick ecology rules, for the scientific prevention of future epidemic in Henan Province Xinyang tick borne diseases provides scientific basis.

Materials and Methods

Ticks Specimen Collection. Based on Xinyang, Henan Province fever with platelet reduce syndrome epidemic area, combined with geography, topography, latitude, elevation, vegetation and climate characteristics, choose Henan Xinyang area in the foothills of the Pingqiao District of Xinyang City, Shangcheng County, Gushi County, Guangshan County as the survey area in each survey area the choice of five villages to carry out fixed regular surveys.

Collection and Investigation Methods of Ticks. We used artificial cloth flag, in the selection of grass or bushes, lots, cloth flag (100cm*100cm, the white flannel made timing (each less than 1 hour), the flag side rubbing sticks, both ends of the rope fixed, ground tile flag, drag rope slowly is moving at a constant speed drag ticks per drag a certain distance (about 5 meters), the cloth flag or drag tick those who tick tweezers pick up into 50ml sterile centrifugal tube and immediately tightened tube cover or plugged plug and ID tags. Each tick is placed in the same tube or does the same number. As flat grassland, drag bunting walking on the grass; if Bush was carrying pole in the bushes and weeds waving back and forth bunting.

The persistent active object pick-up method in domestic animals such as cattle, sheep, dogs and other body surface to pick up the Lord, focus on maintenance of laboratory animal ears, around the eyes, nose and mouth around the neck, armpits, breasts, breast, thigh, scrotum, anus week, perineum, the root of the tail and other parts, hair is long animals need to use hand to touch. Put the ticks in the same tube and number on each of the animals.

The parasitic ticks were collected at the same time, in January 2015 -12 months, the parasitic ticks were collected simultaneously, the parasitic host venous blood was collected, and the serum was removed after centrifugation. The centrifuge tube was placed in the 1.5ml centrifuge tube. A total of 76 sera were collected from dogs, 68 sera from sheep and 37 copies of cow serum.

Pathogen Detection of Ticks Specimen. NA extraction were divided into two groups, tick specimens with sterile distilled water flushing, sterile filter blot moisture, specimens are placed in sterile mortar, adding proper amount of liquid nitrogen rapid freezing, and then adding 500 μ l lysate, rapid ground evenly, after crush use treasure Biological Engineering (Dalian) provided by Takara DNA Extraction Kit, according to the instructions to DNA extraction as a template.

According to literature design fever with platelet reduce syndrome virus, phagocytophilum Anaplasma specific primers for PCR amplification and electrophoresis that sftsv selected highly conserved region of the S segment as the target area, the primer and probe sequences are as follows:

Primer TAAACTTCTGTCTTGCTGGCTCC Forward

Primer TGGCAAGATGCCTTCACCA Reverse

MGB Probe CGCATCTTCACATTGAT Taqman

The PCR gene sequence was used as the target sequence of the 16SrRNA gene, and the specific primers were designed to amplify and electrophoresis.

HGA primer: GE9f:5 '-AACGGATTATTCTTTATAAGCTTGCT-3';

GE10r:5 '-TTCCGTTAAGAAGGATCTAATCTCC-3';

Ehr521:5 'TGTAGGCGGTTCGGTAAGTTAAAG3'.

Amplification instrument for biosye tems7500PCR - sytem Applied (purchased in the United States biosye tems - Applied Company). Reaction conditions: 45 5min, 95 10s, 95 10s, 55 C, 30s, a total of 40 cycles.

The specific PCR products of the tick samples were purified and recovered, and the high throughput sequencing library was constructed by using the recovered product. From GenBank download related sequences, using MEGA6.0 to carry out nucleotide homology comparison.

To collect tick host serum were specified in accordance with the kit for sftsv, HGA antibody detection and nucleic acid detection of serum antibody positive, host serum samples were PCR detection of pathogens, primer sequence and detection steps with ticks were pathogen detection.

Results

Number and Distribution of Ticks Specimen. From January to December in 2015, 1235 ticks were collected from -12 months, including 473 free ticks and 762 parasitic ticks. Identified caught ticks have three, respectively for *Haemaphysalis longicornis*, *Boophilus microplus*, Lahore obtuse marginal ticks; free ticks of *Haemaphysalis longicornis* mainly, a small amount of Lahore *Ornithodoros*; parasitic ticks main *Haemaphysalis longicornis*, a small amount of *Boophilus microplus*. 1235 ticks specimens, a total of 1067 *haemaphysalis longicornis* accounted for only 86.4%, was the dominant species. In Xinyang area of free ticks were 48 manual / h of medicine, ticks collected were 475, average density for 10 (manual / h), the highest for June 14 (manual / h); external environment in April began to tick, 5-8 months tick higher density, September ten days after the sharp drop in the number of, in October, environment disappeared. Among them, the average density was 14 (artificial / hour), and the average density was (artificial / hour), and the average density was 2 (artificial / HR). Choose to capture the fixed point and fixed relative to the domestic host regular tick collection, in Pingqiao District, Shangcheng County, Gushi County, Guangshan County has selected the dog 95 collection to the tick 297 only, average tick density for 3.1 dogs, sheep 81 acquisition to 184 tick, average tick density was 2.3 sheep, cattle 44 acquisition to tick 31 average tick density 0.7 cattle. In March will be able from livestock table to capture the parasitic ticks. In October it basically disappeared. But in November it can still to capture the parasitic ticks in individual animals.

Pathogen Detection Results of Ticks Specimen. In this study, we collected ticks 1235, according to different tick species, status (free, parasitic) and host, divided into group of 54 were specific PCR amplification, gene sequence alignment, with the detection of two kinds of tick borne pathogen specific DNA fragments, as shown in the alignment and fever with thrombocytopenia comprehensive syndrome virus (SFTSV), eosinophilic phagocytophilum were highly homologous.

Host Serum Detection Results. T Body of intangible intangible intangible form of animal parasitic ticks on serum antibody detection were detected in two kinds of pathogen specific DNA fragment, compared to the display and fever with thrombocytopenia syndrome virus (SFTSV), addicted to phagocytic cells have highly homology. The dog serum, 76, found sftsv was positive in 4 copies, the positive rate of 5.26%, addicted to phagocytic cells body 6 positive, the positive rate of 7.89% sheep serum 68 found SFTSV 2 positive, the positive rate of 4.41%, addicted to phagocytic cells body 6 positive, the positive rate was 8.82%; bovine serum 37 that SFTSV 2 positive, the positive rate of 5.41%, addicted to phagocytic cells was positive in 4 copies, the positive rate of 10.81%.

Discussion

Such tick borne spotted fever, encephalitis, Lyme disease, hemorrhagic fever, Q fever, Babesi worm disease tick thorn host biting and sucking blood, bite wounds ulcer formation in to human and livestock harm at the same time, the most important is a variety of transmission of zoonoses.

Which novel bunyavirus induced fever with thrombocytopenia syndrome (Fever, thrombocytopenia and leukopenia syndrome (FTLS)) is since 2009 in mainland China gradually, the occurrence of a new infectious disease and by China's first successfully isolated pathogens, temporarily named as the new bunyavirus. The tick in the important position of natural disease, associated with unique biological characteristics of ticks. Ticks can change the host's habits, so as to extend the range of transmission of the disease, a serious threat to human health and animal husbandry production. Xinyang Henan area is a sub-tropical to warm temperate transition zone, the geographical features of complex and varied, most of the natural ecological environment is covered with weeds and shrubs, special geographical environment is conducive to the breeding of ticks. Farming and animal husbandry is the main pillar industries of the region, has been found in the area found variety of advantages of the tick *Haemaphysalis longicornis* is a small amount of *Rhipicephalus sanguineus*, *Boophilus microplus*, and in this study, we collected ticks were consistent, also with the previous to the region tick fever with platelet reduce syndrome of incentives consistent; the tick distribution regions of high density of Shangcheng County, and previously reported fever thrombocytopenia syndrome of high incidence of epidemic area reduction consistent.

Conclusion

Through this research, it has been cleared about the Henan Province Xinyang fever with thrombocytopenia syndrome the species and distribution of ticks in endemic areas, *Haemaphysalis longicornis* is the absolute dominant species; free ticks in 3-10 months in external environment exists, from May to August are higher density of active period in, in October after, basically disappeared: year of parasitic ticks parasitic on livestock, of which from May to September are the peak density in. Epidemic areas of high tick density for the mall County, the minimum density is close to the city of Pingqiao district. Tick specimens of fever with thrombocytopenia syndrome virus, phagocytophilum nucleic acid was detected in the serum of host fever with thrombocytopenia syndrome virus, phagocytophilum positive nucleic acid detection, suggesting that ticks may be new bunyavirus induced fever with platelet reduction syndrome, eosinophilic phagocyte anaplasmosis the media or the hosts, and the area of fever with thrombocytopenia syndrome, eosinophilic phagocyte anaplasmosis epidemic was correlation between serum infected animal new bunyavirus, *Anaplasma phagocytophilum* positive does not exclude animal spread of fever with thrombocytopenia syndrome, possibility of phagocyte anaplasmosis. We should increase the biological study of ticks in Xinyang area of Henan, and further grasp the biological characteristics of ticks, and provide scientific basis for formulating effective strategies and measures for the prevention and control of diseases.

Acknowledgements

This research was financially supported by the Foundation of Fundamental and Frontier Plan Project of Henan Provincial Department of Science and Technology in 2014 (Grant No. 142300410430).

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