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Identification of Moniezia, Sp in Goat Intestines in Indonesia which Can Impede Goat Productivity

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ABSTRACT

One of the obstacles that can affect the productivity of goats is the threat of parasitic diseases in reared goats. Goats and sheep in Indonesia are susceptible to infestation of this digestive tract parasite due to the influence of the wet tropical climate which is very beneficial for survival and facilitates transmission. Gastrointestinal worms are often found in livestock which can cause a decrease in growth rate and health. Some of the food substances in livestock are consumed by worms, causing tissue damage in animals. This situation can also cause livestock to be more sensitive to various deadly diseases. As a result of intestinal worm infestation can reduce goat productivity. This study aimed to identify Moniezia, Sp in goat intestines in Indonesia. This research method is by using PCR, sequencing, and alignment of amino acids using Blast, the amino acid Moniezia benedeni in bovine intestines was analyzed using Swiss Prot software to determine the three-dimensional structure. The prediction of B cell epitope is conducted using IEDB software. To measure allergen levels and toxicity, this study uses AllerTop and ToxinPred software. The result shows that Moniezia benedini was found in goat intestines. Further research is needed on Moniezia benedeni so that it can be used as a diagnostic kit or vaccine seed.

Keywords: Identification, Goats, Moniezia, Sp, Parasites.

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INTRODUCTION

The consumption of livestock products in Indonesia is growing very rapidly, in line with the development of the population and the increasing awareness of the importance of nutrition [1]. To meet the demand for meat, alternative meat-producing livestock are needed to help support meat needs. One of the potential livestock as an alternative is goats [2].

Goat livestock is one type of livestock that has good development prospects in supplying meat needs. Goats in one breeding period can produce more than one child per birth, making this livestock quite popular with farmers, most of whom are middle to lower class people [3]. The existence of goats cannot be taken lightly because they greatly help the household economy. For breeders, goats can function as savings that can be sold to overcome urgent economic needs [4].

One of the obstacles that can affect the productivity of goats is the threat of parasitic diseases in reared goats. Goats and sheep in Indonesia are susceptible to infestation of this digestive tract parasite due to the influence of the wet tropical climate which is very beneficial for survival and facilitates transmission [5]. Gastrointestinal worms are often found in livestock which can cause a decrease in growth rate and health. Some of the food substances in livestock are consumed by worms, causing tissue damage in animals. This situation can also cause livestock to be more sensitive to various deadly diseases [6].

Digestive tract worm infestations can reduce the productivity of Saburai goats. A single infestation of nematodes in the digestive tract can reduce body weight by 21.71%, cestodes 9.60%, and trematodes by 7.07%. Mixed infection of nemotodes and cestodes resulted in the largest decrease in meat production (41.92%), this could result in a loss of goat and sheep meat production by 17.75% - 24.77% or 3.2 - 4.4 million kg or 7.58 - 10.56 billion rupiah per year. Besides, the disadvantages caused by gastrointestinal worm infestation include reducing production and reproduction performance, as well as reducing feed intake and feed conversion efficiency [7].

Moreover, when the absorption of nutrients is not good, it will inhibit growth will lead to anemia and even death in parasite infestations. Besides, infestation of worm parasites will weaken the body's immunity, so that livestock are more susceptible to infection by other pathogenic diseases and ultimately will cause economic losses [6]. The purpose of this study was to identify Moniezia, Sp in goat intestines in Indonesia.

MATERIALS AND METHODS

DNA extraction

DNA extraction was carried out using Qiagen DNA Mini Kit. Extraction was carried out following the manufacturer's instructions. Monieziaproglottids were placed in the eppendorf tube then added buffer ATL 180 µl, Proteinase K 20 µl, and Lisosim 5 µl, mixed using vortex and mini micro centrifuge. Incubation was carried out at 60 ° C for 120 'using Thermo Stat Plus. The samples in the Eppendorf tube were then mixed using a vortex and mini micro centrifuge for each addition of 200 µl of AL buffer (lysis buffer) and 96% ethanol 200 µl [8].

The sample was then transferred to the QIAamp mini spin column using a micropipette and centrifuge at 8,000 rpm for 1 '. The top of the QIAamp mini spin column was transferred to the collection tube and washed with AW 1 500 µl buffer then centrifuged at 8,000 rpm for 1 '. The upper part was transferred to a collection tube and washed with AW 2 500 µl buffer then centrifuged at 13,000 rpm for 3 '. The upper part was transferred to a collection tube and dried by centrifuge at 13,000 rpm for 1 '. 50 µl of AE buffer was added then incubated at room temperature for 1 'and centrifuged at 8,000 rpm for 1'. The obtained template DNA was stored at freezer temperature -200C [9].

PCR

The identification of Moniezia species was carried out molecularly. DNA fragments (about 875 bp) were amplified using polymerase chain reaction (PCR) using

ITS1F 5.8S rRNA primers (5'-GCTGCTACCCGCATGATGTT-3 '). The 18S rRNA gene sequence M. expansa (GenBank No. KX377890.1) and M. benedeni (GenBank No. AB367792.1) taken from GenBank (NCBI, http: // www.ncbi.nlm.nih.Gov/Entraz). PCR amplification was carried out in a volume of 20 µl for each sample containing 12.5 µl of mastermix (Promega), 0.5 µl distilled water, 1 µl of ITS1F 5.8S rRNA primer, and 5 µl of sample DNA [10].

Pre-denaturation was carried out at 94 ° C for 5 ', followed by denaturation at 94 ° C for 45' ', annealing at 50 ° C for 30' ', extension at 72 ° C for 30', and final extension at temperature. 72 ° C for 5 'repeated 35 cycles. DNA samples and controls containing primer, distilled water, and mastermix were injected in 2% agarose gel in 1x TBE buffer. Electrophoresis was carried out under conditions of 110 V, 70 mA for 30 'and visualized under Ultra Violet light [11]. The amino acid Monieziabenedeni in bovine intestines was analyzed using Swiss Prot software to determine the three-dimensional structure. Prediction of B cell epitope using IEDB software, allergen levels, and toxicity using AllerTop and ToxinPred software [6].

RESULTS AND DISCUSSION

In this study, Monieziabenedeni was found in goat intestines based on PCR and sequencing. Infection from Monieziabenedini can be caused by livestock eating grass that contains mites (mites) that contain infective cysticercoids. Several studies have also reported that the prevalence of mixed infestation by several types of worms is quite high, reaching 90%.

The high rate of mixed infestation can be attributed to the inefficiency of the applied livestock health control methods. Farmers rarely take special measures such as separating sick animals from the group, giving vitamins, giving treatment according to visible symptoms, instead sick animals are allowed to continue to join the group [12]

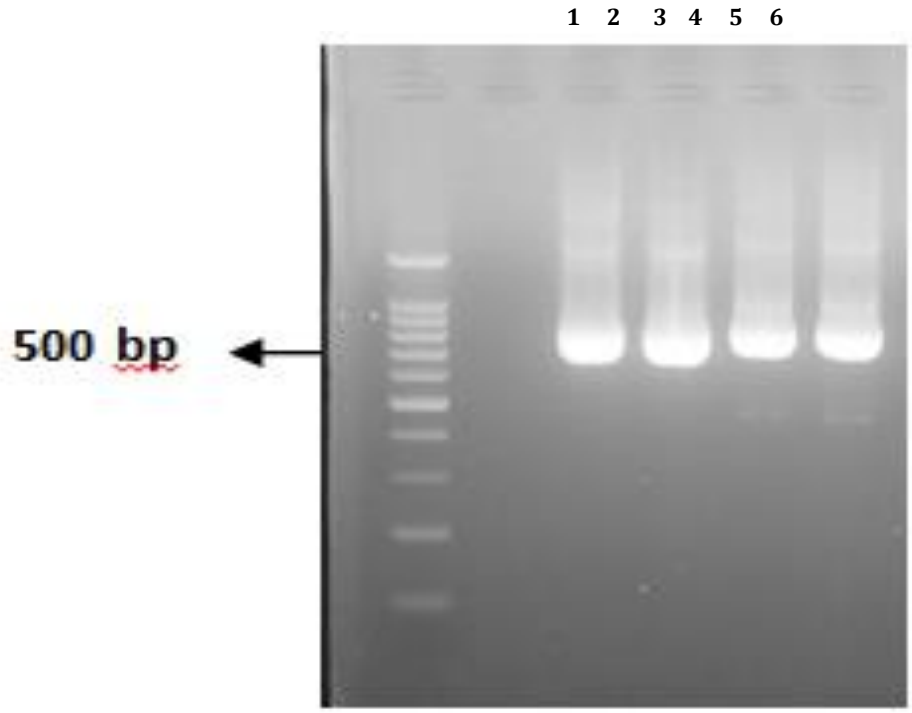

Figure 1. PCR results of Monieziabenedeni in goat intestines
1 = marker; 2 = negative control; 3-6 = samples

The high incidence of gastrointestinal worm infestations in ettawa-bred bean goats, even though the cattle have been locked up in an insulated stage pen is due to the semi-intensive maintenance management applied. The cattle will be removed from the pen and shed in the yard at 10.00 WIT to 15.00 WIT after that the cattle are re-penned.

The high incidence of infestation by nematode worms is strongly suspected to occur especially when eating grass in umbaran / pasture fields that have been contaminated by worm infective larvae. Besides, forage feed in the form of grass is still given when the cattle are in the pen, so this has led to the suspicion that these goats can be infested by worms that have a direct life cycle such as nematode worms.

Sarker et al. state in their study that the method of raising livestock is very influential on the incidence of parasitic infections. If the farmer uses a semi-intensive system by allowing the cattle to forage on their own (the shepherd system) or not at all in the cage (the traditional system) then there is a high chance of being infected with worms. Livestock that are kept intensively (pen system) has a lower risk of infection because the animal feed is given in the pen [13].

Moniezia expansa internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence
Sequence ID: KX377890.1Length: 754Number of Matches: 1

Range 1: 52 to 561GenBankGraphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
776 bits(420)	0.0	482/512(94%)	3/512(0%)	Plus/Plus

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Query  2    TATGCACAATCTTACCTACATACCTCTTGTGTAGGTGTGTGTGTACTTTCGGTGGGGTGC  61
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  52   TATGCACAATCTTACCTACATACCTCTTGTGTAGGTGTGTGTGTACTTTCGGTGGGGTGC  111

Query  62   CTAGTCTGCCTAACACCTGAGATGTGGTATGCCCGCGTGTTCCATACCCGCCGGTCCATA  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  112  CTAGTCTGCCTAACACCTGAGATGTGGTATGCCCGCGTGTTCCATACCCGCCGGTCCATA  171

Query  122  CCCGGGCGGCAGAGCAGTGCACGAGTAGTCCCTCCGCTatgtatgtgtgtgtatgcatg  181
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||  |||
Sbjct  172  CCCGGGCGGCAGAGCAGTGCACGAGTAGTCCCTCCGCTTGTGTGTGTGTGTGT--GTG  229

Query  182  tatgtatgCGGGCTAGGGGTGTGCAAAGAATTATACGTTTGTATGGCTTTCTGTCGCTGT  241
            |||||||||| |||||||||||||| | || | ||||||| |||||||| ||||||||
Sbjct  230  TGCGTGTGCGGACTAGGGGTGTGCAAGGCATAAGACGTTTGGATGGCTTTCAGTCGCTGT  289

Query  242  CGACGCCCTCCTACGCCCCGCCATGTGTCCTGTTATTTTGCATTTATGTTACACTTGTCC  301
            ||| ||| ||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  290  CGAGGCCGTCCTACGCCCCGCCATGTGTCCAGTTATTTTGCATTTATGTTACACTTGTCC  349

Query  302  AGTAATGGTAGAAATAGCAGTAGGTGGTGCGTGGATGTGCAATCTCATTATCATTCTGAT  361
            |||||||||||||||||||||||||||||||||||||||||||||||| ||| ||||||||||
Sbjct  350  AGTAATGGTAGAAATAGCAGTAGGTGGTGCGTGGATGTGCAATCGCATCATCATTCTGAT  409

Query  362  AGGGTGATGTGAATGCTTTTGCCACTGTGTGCCTGCTCTGTCTCTCTCTATCTCCTCGTG  421
            |||||||||||||||||||||||||||||| ||||||| ||| |||||| |||||||||
Sbjct  410  AGGGTGATGTGAATGCTTTTGCCACTGTCTGCCTGCACTGCCTCTCTATATCTCCTCAAC  469

Query  422  -AATGTGCTGGCTATTGTCGTGCACGCGGTCTGGATATGCACACGCCCGAGCGTTAAAGC  480
             ||||||||||||||| | |||| |||||| || |||||||||||||| ||||||||||
Sbjct  470  AAATGTGCTGGCTATTGCCATGCATGCGGTCGGGCTATGCACACGCCCGCGCGTTAAAGC  529

Query  481  GCTATTGCTGtatgtatgtatgtatgtatgtgtgtgt  512
            ||||||||| |||||||||||||||||||||||||
Sbjct  530  ACTATTGCTGTTGTGTGTGTGTGTGTGTGTGT  561
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Figure 2. Alignment Results of Monieziabenedeni amino acids in goat intestines

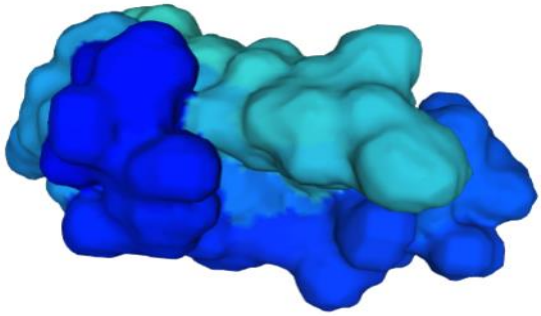

Figure 3. Three-dimensional structure of Monieziabenedeni in goat intestine

Table 1. Prediction of Allergens and Toxicity of Monieziabenedeni in goat intestines

Peptides	Position	Long	Allergen Prediction	Prediction Toxicity
EQKKNEKDVGEIRHRTE	4-20	17	Probable Allergen	Probable Non-Toxin
QDSDFVNYSCNFDSGFKLHSESFIKPMCSSRYTKA PLNTHATVFDNTDKLN	36-86	51	Probable Non-Allergen	Probable Toxin
RGRMKSQV	106-113	8	Probable Allergen	Probable Non-Toxin
PPIKYPSHHY	148-157	10	Probable Non-Allergen	Probable Non-Toxin
KNTSKTLSSHFFTKNKE	167-183	17	Probable Non-Allergen	Probable Non-Toxin

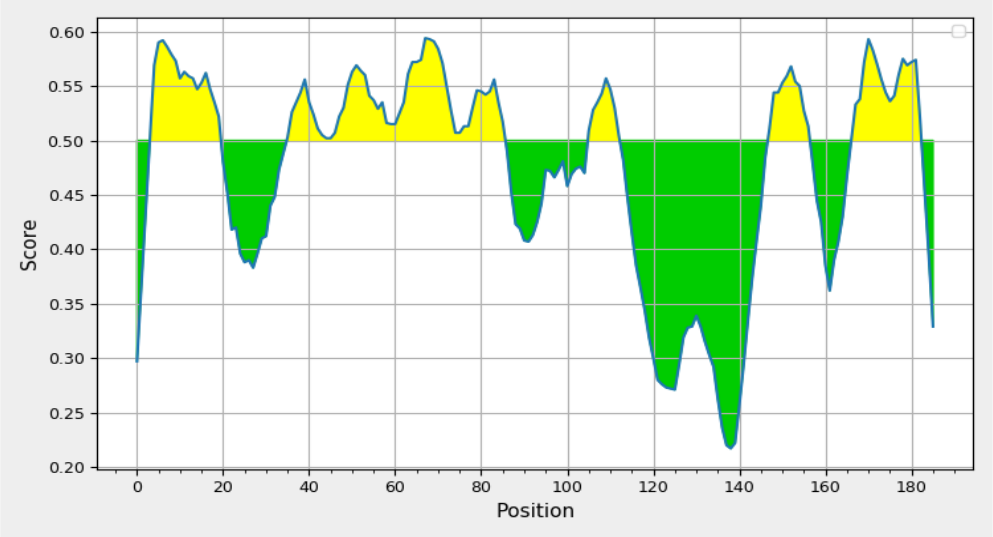

Figure 4. Prediction of Monieziabenedeni B cell epitope in the intestine of goat

Research conducted by Lima (1998) states that differences in cage models are not the main factor causing intestinal nematode infection in goats. The farmer provides forage to the goats every morning in the form of fresh elephant grass without withering process. The fresh forage given is one of the factors causing the high infestation of digestive tract worms in goats due to larval contamination in the forage. Another factor that affects the spread of nematode worms is sanitation and cage hygiene [14].

Litter that is allowed to accumulate in the cage attracts flies and also allows nematode larvae to develop in it. If the skin of the livestock comes into contact with the manure, some worm larvae can enter the body of the livestock. Young cattle are more sensitive to nematode infection than adults [15]. As a result, the immune response in this group of children to limited feed intake will be more serious than that of adult livestock. Areas in Indonesia are generally not clear about the carrying capacity of pasture and the quality of the grass, and the amount of time that cattle spend grazing is also different. This will worsen the health status of the worm-infested livestock [16].

The death rate caused by this gastrointestinal worm infestation occurred in five goats in one month. With an infection by digestive tract worms, there will be disturbances in the form of low growth rates and increased mortality rates. According to the Directorate General of Animal Husbandry (2010), losses due to worm infestation reached 4 billion rupiah per year. This is a disease that can affect productivity, thinness, decreased production power even in severe infections can cause digestive disorders to inhibit the growth of the animal itself. Another effect caused is weight loss due to diarrhea and the effect on the host. [6]

From the research, it was found that non-allergen, immunogenic, and non-toxic B cell-based peptides have the potential to be the seed for M. benedeni vaccine in goats. The development of B lymphocytes requires several stages starting from the progenitor lymphoid cells (common lymphoid progenitor / CLP) to become mature B lymphocytes in the spinal cord. CLP itself comes from hematopoietic stem cells (HSC) which no longer have potential myeloid lines. CLP can develop into T lymphocytes, B lymphocytes, and dendritic cells (DC). B lymphocyte receptors have two types of chains, namely the H chain (heavy chain) and the L chain (light chain) which have various gene loci. Complete immunoglobulin (Ig) has 2 H chains and 2 L chains. In the early stages of cell development, B lymphocytes require recombination of the V, D, J gene loci in the H chain (VDJH) and V, J gene loci in the light chain (VJL).

The recombination of these gene segments makes the B lymphocyte cell assemblies produce antibodies that can recognize more than 5 x 10^{13} different types of antigens. There are three stages of development of B lymphocytes based on the recombination stage and the arrangement of genes forming the L and H chains. They are the pro B cells recombine the D and J gene segments on the H chain, followed by a second recombination in the V segment to combine with the DJ segment [17].

The development of B lymphocytes is regulated by SLC (surrogate L chain) which consists of two proteins, namely λ5 and VpreB. Both of these proteins bind to the μ protein in the H chain and form pre BCR. B cell precursors or pre-B cells are formed from Pro B lymphocytes that express pre BCR on their cell surface. For the subsequent development of B lymphocytes, the Bruton's tyrosine kinase (Btk) gene and the cytokine IL-7 are needed [18].

Mutations in the Btk gene can cause X-linked agammaglobulinemia disease. This BCR signaling pathway in the development of B lymphocytes has received much attention from clinicians. Not only can it be used to differentiate agammaglobulinemia from other types of hypoglobulinemia, but inhibition of this BCR pathway can be used as a promising new therapy [19].

Humoral immune response usually ends with the presence of Immunoglobulin Class Switching to produce a better immune response, because it maintains specificity against antigen but provides a different immune response. At first, B cells only form IgM and IgD, but after an adaptive immune response occurs, B cells secrete antibodies that are adjusted to the type of antigen that enters, so that B cells may produce IgG, IgA, or IgE. In cases of worms [20],

Immunoglobulin Class Switching occurs with the formation of IgE (Abbas &Lichtman, 2011). Immunoglobulin Class Switching occurs because of two important stimuli. The first reason is caused by stimulation by cytokines IL-4 and/or IL-13, while the second stimulation is the bond between CD40 in B cells with CD40L [21].

CONCLUSION

From the results of the research conducted, it was found that Monieziabenedeni in goat intestines. Further research is needed on Monieziabenedeni so that it can be used as a diagnostic kit or vaccine seed.

ETHICAL CLEARANCE

This research process involves animals as a subject that was following the ethical research principle based on the regulation of the research ethics committee. This study implemented the basic principles of ethics of respect, benefit, non-maleficence, and justice.

CONFLICT OF INTEREST

The author guarantees that there will be no report about conflicts of interest in this work.

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