The Effect of Fever on Activity and Vital Signs in Mice (*Mus Musculus*)

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Abstract: Fever is a condition where the body temperature is above normal as a result of an increase in the thermoregulatory center in the hypothalamus. The occurrence of an increase in temperature in animals needs to be measured to ensure the occurrence of fever. Fever will cause changes vital signs and activity patterns in animals. The changes vital signs that were seen included eye temperature, nose temperature, abdominal temperature, rectal temperature, respiratory rate, and activity patterns which were measured for one minute using optovarimex measuring instrument. This study used 24 mice divided into three groups; normal group, group were given DTP-HB vaccine to trigger fever, and group was given NaCL-fis. Each group consisted eight mice of the same sex. Each group will measure eyes temperature, nose and abdomen using thermal camera, then measure rectal temperature using digital thermometer (control temperature) to ensure increase temperature, then calculate respiratory rate for one minute, and measuring activity patterns using the optovarimex for one minute. The research results show; the application of DTP-HB vaccine had an impact on increasing rectal temperature, thermal camera showed not optimal data, because the object was too small. The increase temperature causes the respiratory frequency in mice increase for dissipate heat. Application of physiological NaCl slightly increased respiratory rate but was not significant when compared to feverish conditions. Measurement activity patterns showed that distance traveled (DT) of the fever mice was very small, mice more silent/rested (RT), this was stark contrast to normal treated mice. The conclusion of this study is fever greatly affects decrease in activity pattern of mice and increases the respiratory frequency.

Keywords: fever; respiratory rate; optovarimex, activity

Introduction

An increase in body temperature or commonly called fever is a condition where the body temperature is above normal as a result of an increase in the thermoregulatory center in the hypothalamus. An increase in body temperature or fever is not a primary disease but is a beneficial physiological mechanism in fighting or protecting against infection (Sodikin 2012). An increase in body temperature or fever is a picture of various diseases that are often caused by bacterial or viral infections (Lusia 2015). The occurrence of an increase in temperature in animals needs to be measured to ensure the occurrence of fever. The tools that can be used in measuring body temperature are digital thermometers and thermal cameras. Measurement of body temperature in living things can be done in various parts of the body, namely the armpits, under the tongue (oral), forehead and rectal. The most accurate temperature measurement is in

the rectal area, while measurements in other parts of the body can be confirmed by comparing the temperature obtained with the rectal temperature (Kapti and Azizah 2017). According to Fuadi et al (2015) fever or an increase in body temperature can result in impaired physical activity and decreased intelligence, so that if these factors are seen in animals, prevention can be done earlier.

According to research conducted by Agustin et al.(2017) one of the ingredients that can cause an increase in temperature in animals is the DTP-HB vaccine. The DTP-HB vaccine is a homogeneous colloidal suspension containing pure tetanus toxoid, pure diphtheria toxoid and inactivated B. pertussis, as well as non-infectious pure hepatitis B virus surface antigen, used to prevent diphtheria, tetanus, pertussis and hepatitis B simultaneously (Ahyar and Muzir 2019). Based on the research above, the DTP-HB vaccine was chosen as a stimulus material to trigger an increase temperature in mice. Mice are animals with usage rates as laboratory animals or animal models reaching 40%. Mice have advantages as laboratory animals, namely having a relatively short life cycle, large number of children per birth, easy to handle, and similar production and reproductive characteristics to other mammals (Nugroho 2018). Based on these things, mice were chosen as the model animal in this study.

An increase in temperature or fever can result in various changes in vital signs in the body of mice or other animals, which can lead to abnormal conditions, so research is carried out to determine what vital signs are in the body of animals, especially mice that can be seen when there is an increase in body temperature or fever. .

Methods

This research will be conducted from March 2021 to April 2021. Data collection is carried out in the physiology laboratory of the Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University. The tools used were optovarimex, infrared thermal cam, mouse cage with wood husks, thermometer, cool box, computer. The materials used were 24 mice, DTP-HB vaccine, 0.9% NaCl, as well as feed and drink for mice during the study. The research design used was a randomized block design and each treatment consisted of 8 rats as replicates. Quantitative data from optovarimex observations will be analyzed using One Way Analysis of Variance (ANOVA). Data analysis for thermal cameras was carried out using the Statistical Product and Service Solution (SPSS) software.

The first procedure carried out was the preparation of three cages with wooden husks placed in a perforated box as a place for air circulation, the cages were placed in an air-conditioned room with a temperature of 20-230C, then the division of 24 mice into three groups where each group contained 8 mice, the groups were divided into the normal group, which was not given the DTP-HB vaccine injection treatment, then the mice group which was given the DTP-HB vaccine injection treatment and finally the mice group which was given 0.9% NaCl. The three groups of mice were placed in different cages.

Treatment mice were given treatment one by one, not done at once directly, to avoid negligence that could affect the data. The first treatment was injection of DTP-HB vaccine in the treated group of mice, DTP-HB vaccine was injected intramuscularly in the thighs of mice with a dose of 0.1 mL/100g BW, after the vaccine injection, the mice were placed in a temporary container. to wait for the vaccine to react for 30 minutes, then the rats were put into the optovarimex device and allowed to stand for 1 minute to adapt to the new environment, activity testing using optovarimex was carried out for 1 minute which then the results would be recorded in a special application on the computer screen monitor. After testing with the activity of the optovarimex device, vital signs were tested, such as rectal temperature, surface temperature and respiratory rate. Rectal temperature testing is carried out with a thermometer attached to the rectal part of the mouse, for the surface temperature data is taken using a thermal cam that is fired into the head and body surface of the mouse, after that the respiratory rate is calculated for 15 seconds with the inspection method for the movement of the thorax and abdomen. will be multiplied by four times to determine the respiratory rate for one minute.

The above procedure was repeated for the treatment in the group of mice that were injected with DTP-HB vaccine, while for the normal group, the tests carried out remained the same except that the DTP-HB vaccine was not injected first, the treatment using 0.9% NaCl was carried out with the same procedure, namely with intramuscular injection of 0.9% NaCl at the beginning of the treatment.

Result

1. One-Way ANOVA Test Results Against The Temperature Calculation

Table 1. The results of the one-way ANOVA test against the calculation of the normal treatment temperature, DTP-HB and 0.9% NaCl.

Treatment		Normal	DTP-HB	NaCl 0.9%	Average	SIG
Temperature	**Eye**	38.75 ± 0.63^{A}	38.75 ± 0.33^{A}	37.7 ± 0.28^{B}	38.4±0.66	0
	Nose	34.25 ± 0.87^{A}	37.36 ± 0.65^{B}	36.75 ± 0.46^{B}	36.12±1.5	0
	Abdomen	36.39 ± 0.61^{A}	34.05 ± 1.28^{B}	32.95 ± 0.5^{C}	34.46±1.7	0
	Rectal	37.94 ± 0.29^{A}	38.59 ± 0.16^{B}	37.78 ± 0.26^{A}	38.10±0.4	0

Note: SIG=Significance value, P>0.05= the data is not significantly different, P<0.05= the data is significantly different. Superscript letters indicate subunit differences between lines.

The table above is the results of one-way ANOVA test for eye, nose, and abdominal temperatures measured using an infrared thermal camera. The significance value of each treatment is presented in the rightmost column. Taking temperature in the eye region of normal mice and injected with DTP-HB vaccine showed the same results, which was ±38.75 higher than the administration of 0.9% NaCl which showed a temperature result of ±37.7.

Temperature measurements in the eye region in each treatment had significantly different results (P <0.5). The temperature in the nasal region had the highest result in the DTP-HB vaccine injection treatment of ±37.36 with a significantly different value (P<0.5). In the large abdominal region, the highest temperature was seen in the normal treatment ±36.39 with a significantly different significance value (P<0.5). In the rectal region, the highest measurement value was seen in the DTP-HB vaccine injection treatment with a value of ±38.59 with a significantly different significance value (P<0.5).

2. One-Way ANOVA Test Results Against The Respiratory Rate Calculation

Table 2. The results of the one-way ANOVA test against the calculation of the respiratory frequency of normal treatment, DTP-HB and 0.9% NaCl.

Treatment	Normal	DTP-HB	NaCl 0.9%	Average	SIG
respiratory rate	148.5±6.21A	184±7.70B	157.5±4.24C	163.33±16.5	0

Note: SIG=Significance value, P>0.05= the data is not significantly different, P<0.05= the data is significantly different. Superscript letters indicate subunit differences between lines.

Measurement of breath frequency showed the highest result in the DTP-HB vaccine injection treatment, which was ±184/minute with a significantly different value (P<0.5).

3. One-Way ANOVA Test Results Against The Activity Calculation

Table 3. The results of the one-way ANOVA test against the calculation of normal treatment activity, DTP-HB and 0.9% NaCl.

Treatment		Normal	DTP-HB	NaCl 0.9%	Average	SIG
Activity	DT	484.88 ± 186.65^{A}	18.13 ± 23.90^{B}	135.88 ± 48.36^{C}	212.96±229.08	0
	RT	21 ± 12.88^{A}	57.88 ± 2.85^{B}	45.88 ± 2.94^{C}	41.58±17.37	0
	AT	39 ± 12.88^{A}	1.13 ± 1.64^{A}	14.13 ± 2.94^{B}	18.08 ± 17.65^{C}	0

Note: DT=Distance Time, RT=Resting Time, AT=Ambulatory Time, SIG=Significance value, P>0.05= data is not significantly different, P<0.05= data is significantly different. Superscript letters indicate subunit differences between lines.

In measuring the activity of mice using optovarimec, the highest DT (Distance Traveled) results were shown in normal mice at a distance of ±484cm, while the lowest distance was shown in mice injected with DTP-HB vaccine at a distance of ±18.13cm, with significantly different values (P<0.5) . Data RT (Resting Time) of mice injected with DTP-HB vaccine was the highest result with a long resting time (silent) for ±57.88 seconds with a significantly

different value (P<0.5). The highest AT (Ambulatory Time) data was in normal mice with a long time of movement in place for ±39 seconds with a significantly different significance value (P<0.5).

Discussion

This study seeks to determine the effect of increasing temperature, especially during fever in mice (mus muscullus). Based on the taxonomy of mice belonging to the family pupilae, genus Mus and species M musculus, mice in Indonesia are divergent from mice in Southwest Asia (Kartika et al. 2013). Septiawan et al. 2014 states that the normal temperature in the body of mice is 37.40C, then when the body temperature exceeds 37.40C the mice have a fever. Normal rectal temperature in mice will be 0.50C higher than normal body temperature (Lisdiyanti 2008).

Measurement of body temperature in living things can be done in various parts of the body, namely the armpits, under the tongue (oral), forehead and rectal. The most accurate temperature measurement is in the rectal area, while measurements in other parts of the body can be confirmed by comparing the temperature obtained with the rectal temperature (Kapti and Azizah 2017). This study obtained the measurement results of rectal temperature in normal treatment, namely 37.940C, this result is in accordance with the literature which states that normal rectal temperature is 0.50C higher than normal body temperature (37.40C). Measurement of rectal temperature after the injection of DTP-HB vaccine increased to 38.590C, which indicated that the mice were already in a feverish state because it was higher than their normal temperature (37.40C+0.50C). In the treatment of 0.9% NaCl injection, the rectal temperature obtained was still in the normal temperature range of 37.780C. Rectal temperature data on DTP-HB vaccine injection treatment indicates that the vaccine is effective for increasing body temperature in mice because after DTP-HB vaccine injection the rectal temperature in mice exceeds its normal limit.

Examination of the temperature of the eye region using a thermal camera showed the similarity between normal conditions and the examination after being given the DTP-HB vaccine, which was 38,750C, according to Schaefer et al. 2004 in Santoso et al. 2020 The increase in core temperature is in line with the increase in temperature in the eye region. The results of the examination of the eye region temperature in mice that were given the DTP-HB vaccine have shown conformity with the literature but the results of normal examinations have quite a large difference compared to the results of the examination of rectal temperature, this can occur because the eye size of mice is smaller than other test animals such as cattle and anoa in other studies so that the accuracy of measurements in the eye region is very prone to errors. Examination of the nasal region showed the highest average result after the animals were injected with the DTP-HB vaccine, which was 37.360C.

Temperature value after vaccination which did not differ much from the rectal temperature was in accordance with the statement of Sellier et al. 2014 that the nose temperature is part of the thermal windows, so it has a temperature close to the core temperature, while the average nose

temperature is normal and the administration of NaCl 0.9% shows a significant difference when compared to the rectal temperature, this is not in accordance with the statement of Sellier et al. al.2014 because the nose temperature is not close to the core body temperature. According to Knížková et al. 2012 a decrease in the thermogram (the result of the camera's thermal image pattern) can be caused by wetness and the presence of dirt on that part of the body. Water that sticks to the surface of the body greatly affects the surface temperature because water is able to absorb the heat released by the body (Adhianto et al. 2015). This decrease in heat occurs due to heat conduction from the body to the water (Hall 2011).

Average abdominal temperature shows significant results when compared to rectal temperatures, this is because the abdominal region has a thick layer of skin and fat, so that the heat released tends to be less. According to Soerensen & Pedersen (2015) in Santoso et al. (2020) the body part of the animal's surface that has a thick layer of fat will be included in the non-thermal windows, so it will show a lower surface temperature value when compared to the core temperature. Another factor is the abdominal position of the mice, either standing or sitting very close to the ground surface, which tends to have a lower temperature than body temperature, so that it can affect the measurement of abdominal temperature.

According to Musser et al (2008) the normal respiratory frequency in mice is 90-163/minute. Measurement of respiratory rate in normal conditions shows the results of 148/minute which is still within the normal range. Measurement of the respiratory rate after administration of the DTP-HB vaccine showed results of 184/minute, these results had passed the normal range of 163/per minute, the state of fever after administration of the DTP-HB vaccine affected the respiratory rate of mice. Increasing the frequency of breath is one of the efforts to dissipate heat through the air, the metabolic rate causes body heat to increase and even reaches its highest point so that it triggers thermoregulation in the body, one of the body's responses is to increase the frequency of breath with the aim of removing heat from the body faster (Wuryanto et al. 2010). Administration of 0.9% NaCl also caused an increase in respiratory rate, but not significantly, this was presumably due to trauma or anxiety that arose when 0.9% NaCl was injected in mice. Anxiety that occurs will be responded to by several changes in the body, especially in vital signs, changes that occur can be an increase in respiratory frequency, pulse and blood pressure (Arini et al. 2017).

The use of optovarimec to detect the movement activity of mice in each treatment will show the results of DT, RT and AT. DT (distance traveled) is the distance traveled by mice or the displacement of mice from one place to another. The results of measuring the distance traveled by mice in this study showed the highest results during normal conditions, namely 484.88 while the shortest distance occurred after DTP-HB vaccine injection, which was only 18.13. Administration of 0.9% NaCl showed a result of 212.96. The results were very significantly different between normal conditions and after DTP HB vaccine was injected due to the influence of fever on activity, this is in line with the statement of Fuadi et al. (2015) which states that fever or an increase in body temperature will result in impaired physical activity and a decrease in intelligence so that it can affect the activity of mice. Another factor that can affect activity is due to the treatment of DTP-HB vaccine injection and intramuscular injection of

0.9% NaCl in the hamstrings of mice, the injection causes pain in the locomotor organs, causing a decrease in activity but not significant, this can be noticed after the injection of 0.9% NaCl which shows the mileage of 212.96. A state of significantly decreased activity occurred after administration of the DTP-HB vaccine caused by fever.

RT (resting time) or rest time is the time it takes mice to rest (quiet) this is needed by mice to save energy. The measurement results show that the administration of the DTP-HB vaccine has a very large effect on the resting time of the mice, this is due to the fever which results in a decrease in the activity of the mice so that the mice will spend a lot of time resting. When at rest the body will release about 25% of body heat or what is called evaporation, whereas when doing a lot of activities will affect the increase in body temperature, if the body cannot release heat through evaporation, the body temperature will continue to increase (Graha 2010). Indirectly resting is the body's response as an effort to reduce body temperature so that the longest results from RT observations occurred after the administration of the DTP-HB vaccine due to fever in mice.

AT (ambulatory time) is the time measured when the mice start a movement such as grooming, feeding, defecation or urination without changing location. The measurement results showed that the highest AT time occurred during normal conditions, while the lowest was after DTP-HB vaccine was injected. This indicates that fever also affects minor activities such as grooming, defecation or other movements without displacement. In accordance with the statement of Graha (2010), moving will cause an increase in temperature in a living being. The results show the response of the body of mice to reduce body heat by reducing body movement or activity, so that the lowest AT result is shown after the injection of the DTP-HB vaccine, which is only 1.13.

Conclusion

Fever is very influential on decreasing the activity pattern of mice and increasing the frequency of breathing in order to dissipate heat. Giving DTP-HB vaccine will increase the core temperature in mice. The small size of mice affects the measurement of the thermal camera but does not affect the measurement of core temperature using a digital thermometer. Suggestions that can be conveyed from this study are to continue further research by adding measurements of heart rate with more repetition of data collection so that the results of the study can be used as a reference for detecting fever more easily in animals.

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