SIMPLE METHOD LARYNGOSCOPE ON RAT INTUBATION

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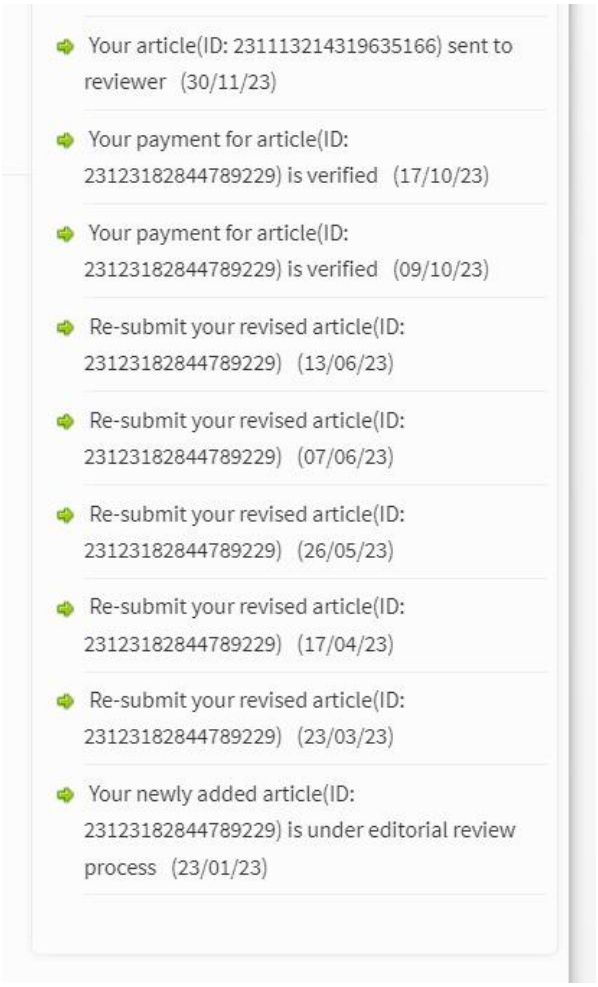

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Authors: **Prananda Surya Airlangga; Nancy Margarita Rehatta; Soetjipto; Eddy Rahardjo; Widjiati; Yopie Wiguna**

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Simple Method Laryngoscope on Rat Intubation

Prananda Surya Airlangga,[1*] Nancy Margarita Rehatta,[1] Soetjipto,[2] Eddy Rahardjo,[1] Widjiati,[3] Yopie Wiguna[1]

[1]Department of Anesthesiology and Intensive Therapy, Faculty of Medicine, Airlangga University, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, 60131
[2]Department of Physiology and Medical Biochemistry, Faculty of Medicine Airlangga University, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, 60131
[3]Department of Veterinary Science, Faculty of Veterinary Medicine Airlangga University, Surabaya, Indonesia. 60115

Corresponding author:
Prananda Surya Airlangga
E-mail: prananda-s-a@fk.unair.ac.id
Department of Anesthesiology and Intensive Therapy, Faculty of Medicine, Airlangga University, Dr. Soetomo Teaching Hospital. Jl. Mayjen Prof. Dr. Moestopo No.47, Pacar Kembang, Tambaksari, Kota Surabaya, Jawa Timur 60132, Indonesia.

Abstract
Background: In research using rats as experimental animals that involve surgery and anesthetic procedures, it is often necessary to administer inhalation anesthetics and patency of the airway. In this condition, intubation in rats is required. Endotracheal intubation in rats is quite difficult because the size and structure of the airway is very small and smooth and there is no specific instrument designed for intubation in rats.
Methods. The experimental animal used in this study was adult male Wistar rats weighing 250-350 grams. The intubation equipment used is a commercially available otoscopy which has a light source and a camera that can be displayed on a mobile phone screen. The end of the otoscopy is a modified blunt-tipped plastic blade. The endotracheal tube that is used was a intravenous catheter size 16 G which was directed using an introducer made of blunt-tipped wire. The rat is positioned on its back dorsally with its head upward.
Results. In this study, 32 rats were intubated. Fourteen of the 32 intubated rats were alive, awakened from anesthesia and extubation. There were no complications such as stridor or voice disturbances after this intubation procedure. The other eighteen rats were not extubated alive because this was part of the treatment in another study. Of the 32 rats that were subjected to intubation, 2 rats failed to intubate due to spasm and bradycardia.
Conclusion. Methods that were given in this paper can be alternatives for rat intubation.
Keywords: intubation, laryngoscope, rats, video

INTRODUCTION

In conducting invasive clinical trials, the use of small experimental animals in laboratories such as rats is very important to achieve this goal. Research which involves surgery and research in the field of surgery and anesthesia, often requires inhalation anesthetic procedures and airway patency[1]. Giving inhalation anesthetics can be done in the inhalation room (gas chamber) or using a special hood for rats,[2–4] however, to administer anesthetic gas in measured concentrations, as well as to maintain airway patency, endotracheal intubation in rats is required. Endotracheal intubation in rats is quite difficult because the size and structure of the airway is very small and smooth (oral cavity, epiglottis, vocal cords) and there is no specific instrument designed for intubation in rats. These difficulties often result in structural damage

to the tone path and death of experimental animals. In this paper, the researchers describe a technique that can be used to perform endotracheal intubation in rats.

METHODS

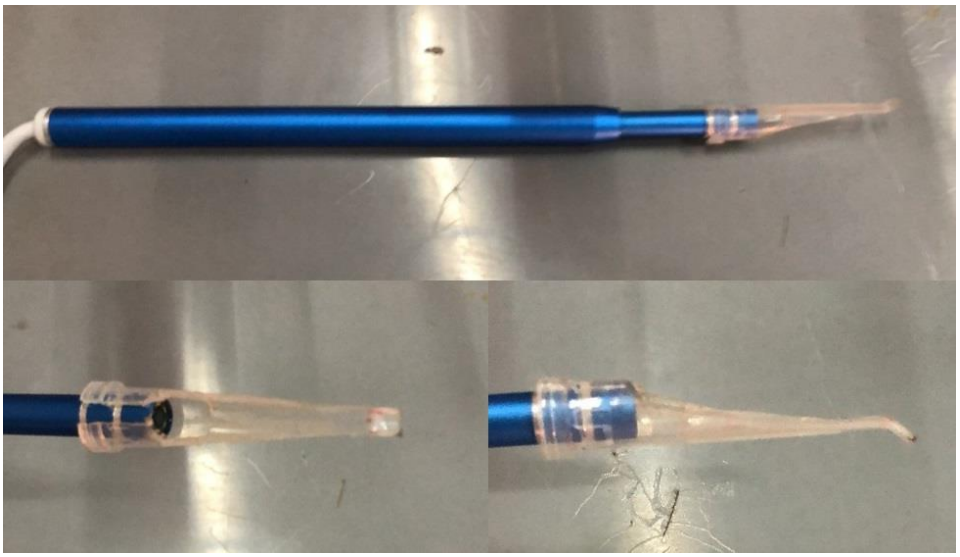
Experimental animal

The experimental action and protocol have been approved by the Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, Surabaya. The experimental animal used in this study was adult male Wistar rats weighing 250-350 grams. All rats are placed in well-ventilated cages with sawdust mats and have access to clean water and animal feed. The room temperature is kept between 20-24^{o} C. All rats used in this study were the subjects of another study regarding hypothermia in anesthetized rat patients.

Equipment used

The intubation equipment used is a commercially available otoscopy which has a light source and a camera that can be displayed on a mobile phone screen. The end of the otoscopy is a blunt-tipped plastic blade originating from the modified 1 ml syringe cap (Figure 1).

Figure 1: Camera otoscopy with modified plastic tip.



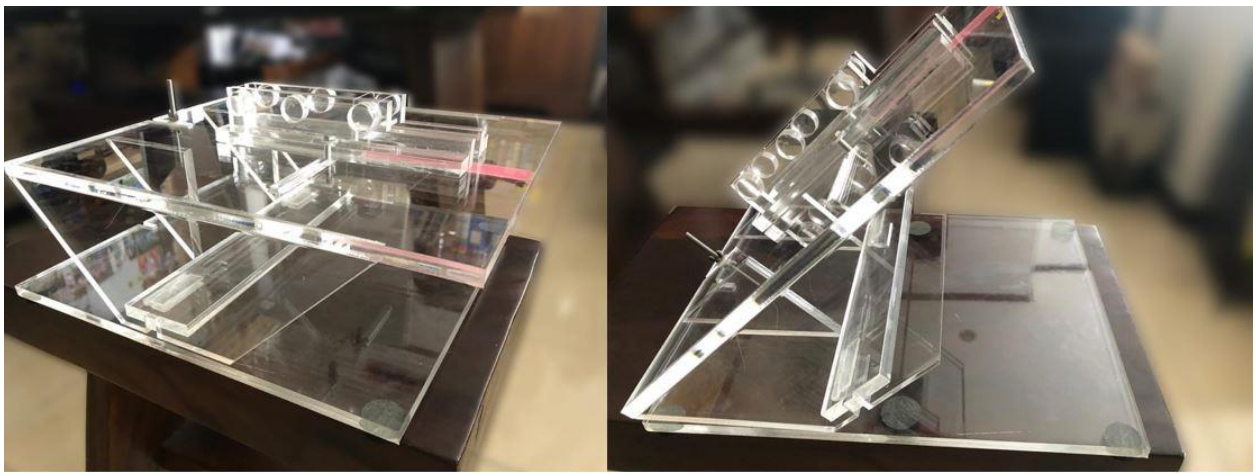
This tip can be made of a syringe cap that is curved using heat, is smoothed, and curved at the tip to prevent injury to the rat's airway. The power source in this otoscopy is obtained from a mobile phone which also functions as a screen to display images. As a tool to reveal the tongue, a cotton swab was used to roll the rat's tongue outwards. The endotracheal tube used was a 16 G intravenous catheter which was directed using an introducer made of blunt-tipped wire (Figure 2). The introducer uses a blunt-tipped wire which is glued to a 1 ml syringe for ease of use.

Figure 2: The endotracheal tube uses a 16 G intravenous catheter

Intubation procedure

The rats were anesthetized with Ketamine (50 mg/kg body weight) and Xylazine (5 mg/kg body weight) by intramuscular injection into the thigh muscle. After the rats were sedated and limp, an ECG monitor electrode was placed on the chest and thighs and peripheral oxygen saturation in the tail. The rat is positioned supine dorsally (half lying 30-45^{o}) with the head above. As fixation, the upper incisors of the rats were treated using rubber on an intubation base. A tilt-changeable base is used as the basis for the action of intubation (Figure 3).

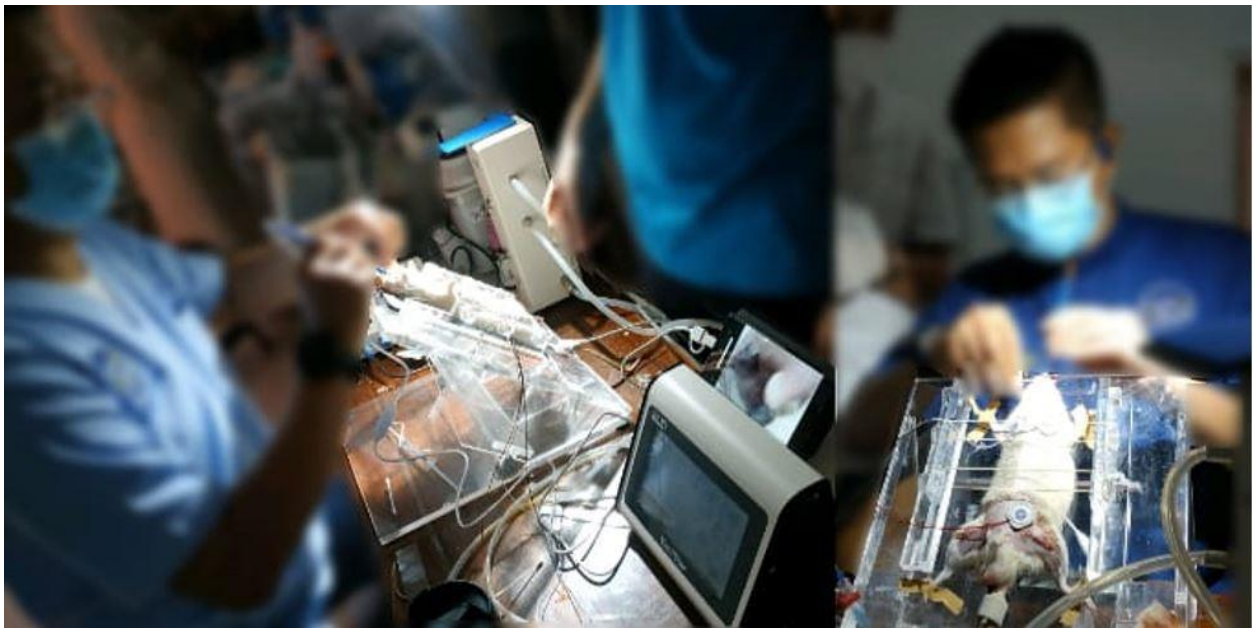
Figure 3: Tilt-adjustable acrylic intubation base



The intubation operator opened the rats' mouth with their right hand and rolled their tongue out using a cotton swab. The operator's left hand enters the intubation device. The airway is visualized on a telephone screen respectively the cavity oropharynx, then the larynx of the vocal cords. The vocal cords were moistened with 2% lidocaine local anesthetic using the tip of a cotton swab. After the vocal cords are anesthetized by fear of opening the vocal cords, the endotracheal tube (16 G intravenous catheter) is inserted using a conveyor. After the catheter is inserted into the trachea, the insert cannot be removed, and the intubation device is removed

from the oral cavity. Then the endotracheal tube is connected to the oxygen source. The position of the operator when performing the procedure can be seen in Figure 4.

Figure 4: The position of the intubation operator when performing the action. Note that the anvil is tilted 45º during action.



The correct placement of the tube was confirmed by auscultation using a neonatal stethoscope and the movement of the rat's chest was symmetrical and rhythmic. The endotracheal tube was fixed in the mouth of the rats using a rubber band. This study used a small animal ventilator as a source of oxygenation and ventilation. Gaseous anesthesia is given using an isoflurane vaporizer.

RESULTS

In this study, 32 rats were intubated. The intubation procedure can be performed simply and quickly. The time taken for intubation ranges from 1 minute. It takes practice and habituation, two anesthetists who perform the intubation procedure need 2-3 experiments on rats before reaching the ability to intubate the rats smoothly. Fourteen of the 32 intubated rats were alive, awakened from anesthesia and under extubation. There were no complications such as stridor or voice disturbances after this intubation procedure. The other eighteen rats were not extubated alive because this was part of the treatment in another study. Of the 32 rats that were subjected to intubation, 2 rats failed to intubate due to spasm and bradycardia. Both occurred in the rats that were the initial sample and could result from a lack of operator experience and skills.

DISCUSSION

In this study, the authors describe the use of intubation equipment in rats that can be easily obtained and modified. The use of a camera with a light source greatly facilitates visualization of the airway structure of very small rats and avoids damage and trauma to the airway structure. By using this technique, generally the intubation procedure can be performed by one operator, sometimes requiring the assistance of one assistant to fix the tongue or help to get instruments.

There is literature describing the method and use of endoscopic equipment and video laryngoscopy specifically for intubation of small animals but these are generally expensive and difficult to obtain[5–9]. The technique used in our study was previously described in research by

Clary in 2003. Clary uses a technique similar to intubation in humans where the operator is right-handed, the scope is operated using the left hand, while the right hand uses cotton buds and inserts the endotracheal tube (ETT) previously attached to the stylet[10]. The advantage of this method is that it's similar to human intubation techniques, thereby simplifying the learning curve for operators with medical backgrounds. Clary's research uses special video-endoscopy equipment.

The research by Balzer in 2020 used commercially available ear wax removal kits and is very similar to the author's research[11]. The advantage of using this tool is that it does not require special veterinary equipment which can be very expensive and difficult to obtain[12,13]. The blade of ear wax removal is analogous to the blade of Miller's laryngoscope used in humans. The study by Samsamshariat used a Miller laryngoscope size 0 and a small diameter wire as a stylet which is commonly used for intubation in neonates and premature infants[14]. The method by Samsamshariat provides 100% success rate and no serious complications.

Another research used a blind technique, which does not use direct visualization tools for the larynx. The drawbacks of the blind technique require several attempts to successfully position the endotracheal tube in the correct position, putting it at risk of traumatizing the very fine structures of the rat airway[15–17]. Research by Zheng in 2020 compared 3 techniques for intubation in mice, namely blind technique, transillumination and direct incision in the trachea. Zheng's research found that the blind technique outperformed other techniques with shorter intubation times, fewer trials and less complications of rat airway damage than other methods[18].

The foundation used in this study uses modified acrylic so that the rat can be positioned supine, properly and safely fixed so that they do not move and can be tilted so that the intubation action can be more ergonomic for the operator. The tiltable rat intubation platform is made using acrylic and is a modification of the Intubation Platform for Rat (IPR) produced by Penn-Century™. The IPR manufactured by Penn-Century™ is discontinued. This acrylic base has been described in other studies. Research by Rendell in 2014 as part of a thymectomy study in adult rats using an acrylic foundation[19].

In this study, the authors applied topical lidocaine to the larynx and vocal cords. Two percent of Lidocaine was applied to the vocal cords and larynx using the tip of a cotton swab moistened with local anesthetic. In the authors' observations, topical application of 2% lidocaine gave a good effect (vocal cords open, decreased airway reflex, and prevented laryngeal spasm) after waiting for its onset for 60 seconds. There were no side effects or toxicity symptoms in the rats given topical anesthesia. The use of topical anesthetics for intubation in rats was also mentioned in the study by Rivard[2].

The use of plastic and cotton to manipulate the airway of rats in this study aims to reduce the risk of trauma and complications. The tip of the plastic blade in this study was made by modifying a 1 mL syringe needle cap which was split and formed a curved tip and smoothed the edges to minimize trauma due to friction. In the future, the use of 3-dimensional printers to print better blades will be possible to provide more ergonomic equipment and minimize injury to the rat's airway[20].

When intubating rats, one of the factors' influencing visualization is the technique to expose the rat's tongue. We found that using dry cotton swabs to roll the rat's tongue out was the most effective method. The method of exposing the tongue using dry cotton swabs was also mentioned in another study and was said to provide a good visualization of the laryngeal

structure of rats[2,21]. Research by Rendell used tweezers to expose the rat's tongue, but this technique requires assistance and the likelihood of injury to the rat's tongue is bigger[19]. To confirm the placement of ETT in the trachea, the authors used the auscultation technique as in general human intubation. The stethoscope used is a stethoscope for neonates. The author's experience of this technique is quite reliable and found no false routes that were not detected. Breath sounds are heard in both chest fields after the ETT is connected to the ventilator. Several other studies used a drop of water in an infusion tube connected to the ETT, the breath air will move water droplets that would not be found in the event of esophageal intubation[22–24]. This method requires the rat to continue to breathe spontaneously.

The complications that the authors found in this study were spasm and bradycardia. Some of the factors that may influence the occurrence of complications are the operator's ability that has not been well honed, which makes intubation attempts to be done several times and anesthesia that is too shallow, causing spasm in the rat's airway. In the literature, complications accompanying intubation in rat include hypersalivation and plugging (due to excessive saliva production), edema and airway bleeding (due to repeated intubation trials), spasm (due to inadequate anesthesia) and anesthetic drug overdose[2,5,23]. Injury at the microscopic level after oral intubation in mice was described by Jahshan in 2018 who found that epithelial and ciliary damage to the airway was found in all rats. who were intubated primarily on ETT use for more than 6 hours[25].

The main advantages of our method are simplicity and relatively low cost to assemble the required tools. The author's experience in using this tool is also straightforward and only takes a short amount of time to achieve the learning curve needed to master it. The operator's background is an anesthetist who is accustomed to performing intubation in humans, where there are similar structures in the airways of humans and rats so that this eases the learning curve. More training and time may be required for researchers from different professional backgrounds to reach the required level of proficiency.

CONCLUSION

The intubation in mice described in this study uses equipment that is inexpensive and easy to obtain, easy to study and has a high success rate.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

PSA, SS, and NMR have developed the research protocol and performed the experimental activities. ER and PSA reviewed and analysed the experimental findings. PSA, WW and YW proposed the ethical application, drafted the manuscript, and submitted to the journal. All authors contributed and gave comments on the research work.

ETHICAL CLEARANCE

The experimental action and protocol have been approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya Indonesia with the number No. 2.KE.015.02.2020.

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Authors: **Prananda Surya Airlangga; Nancy Margarita Rehatta; Soetjipto; Eddy Rahardjo; Widjiati; Yopie Wiguna**

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Paper Title: Simple Method Laryngoscope on Rat Intubation

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With reference to your article titled **'Simple Method Laryngoscope on Rat Intubation'** Author by **Prananda Surya Airlangga, Nancy Margarita Rehatta, Soetjipto, Eddy Rahardjo, Widjiati, Yopie Wiguna**. We wish to bring to your kind notice the following:

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SIMPLE METHOD LARYNGOSCOPE ON RAT INTUBATION

Prananda Surya Airlangga,[1*] Nancy Margarita Rehatta,[1] Soetjipto,[2] Eddy Rahardjo,[1] Widjiati,[3] Yopie Wiguna[1]

[1]Department of Anesthesiology and Reanimation, Faculty of Medicine, Airlangga University, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, 60131
[2]Department of Physiology and Medical Biochemistry, Faculty of Medicine Airlangga University, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, 60131
[3]Department of Veterinary Science, Faculty of Veterinary Medicine Airlangga University, Surabaya, Indonesia. 60115

Corresponding author:
Prananda Surya Airlangga
E-mail: prananda-s-a@fk.unair.ac.id
Department of Anesthesiology and Reanimation, Faculty of Medicine, Airlangga University, Dr. Soetomo Teaching Hospital. Jl. Mayjen Prof. Dr. Moestopo No.47, Pacar Kembang, Tambaksari, Kota Surabaya, Jawa Timur 60132, Indonesia.

Abstract
Background: In research using rats as experimental animals that involve surgery and anesthetic procedures, it is often necessary to administer inhalation anesthetics and patency of the airway. In this condition, intubation in rats is required. Endotracheal intubation in rats is quite difficult because the size and structure of the airway is very small and smooth and there is no specific instrument designed for intubation in rats.
Methods. The experimental animal used in this study was adult male Wistar rats weighing 250-350 grams. The intubation equipment used is a commercially available otoscopy which has a light source and a camera that can be displayed on a mobile phone screen. The end of the otoscopy is a modified blunt-tipped plastic blade. The endotracheal tube that is used was a intravenous catheter size 16 G which was directed using an introducer made of blunt-tipped wire. The rat is positioned on its back dorsally with its head upward.
Results. In this study, 32 rats were intubated. Fourteen of the 32 intubated rats were alive, awakened from anesthesia and extubation. There were no complications such as stridor or voice disturbances after this intubation procedure. The other eighteen rats were not extubated alive because this was part of the treatment in another study. Of the 32 rats that were subjected to intubation, 2 rats failed to intubate due to spasm and bradycardia.
Conclusion. Methods that were given in this paper can be alternatives for rat intubation.
Keywords: intubation, laryngoscope, rats, video

INTRODUCTION

In conducting invasive clinical trials, the use of small experimental animals in laboratories such as rats is very important to achieve this goal. Research which involves surgery and research in the field of surgery and anesthesia, often requires inhalation anesthetic procedures and airway patency[1]. Giving inhalation anesthetics can be done in the inhalation room (gas chamber) or using a special hood for rats,[2–4] however, to administer anesthetic gas in measured concentrations, as well as to maintain airway patency, endotracheal intubation in rats is

required. Endotracheal intubation in rats is quite difficult because the size and structure of the airway is very small and smooth (oral cavity, epiglottis, vocal cords) and there is no specific instrument designed for intubation in rats. These difficulties often result in structural damage to the tone path and death of experimental animals. In this paper, the researchers describe a technique that can be used to perform endotracheal intubation in rats.

METHODS

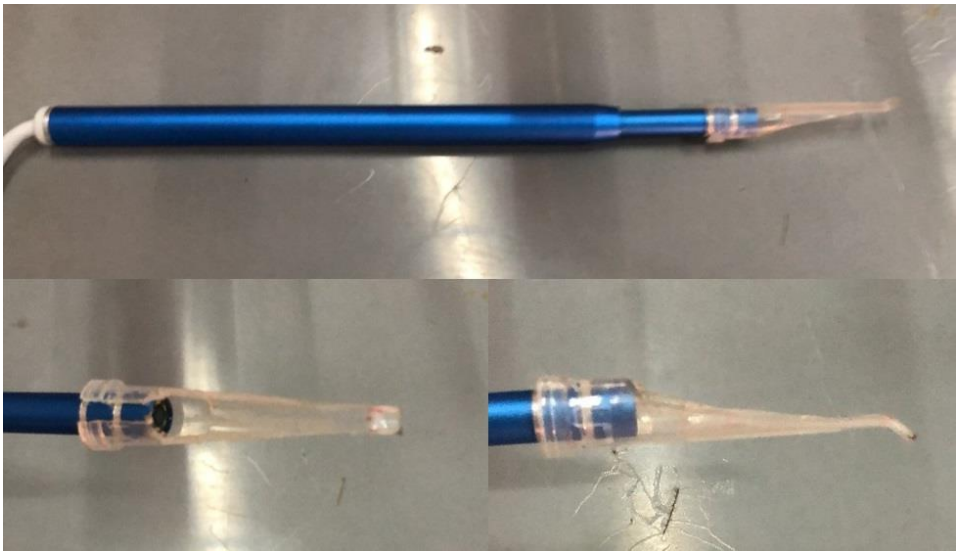
Experimental animal

The experimental action and protocol have been approved by the Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, Surabaya. The experimental animal used in this study was adult male Wistar rats weighing 250-350 grams. All rats are placed in well-ventilated cages with sawdust mats and have access to clean water and animal feed. The room temperature is kept between 20-24^{o} C. All rats used in this study were the subjects of another study regarding hypothermia in anesthetized rat patients.

Equipment used

The intubation equipment used is a commercially available otoscopy which has a light source and a camera that can be displayed on a mobile phone screen. The end of the otoscopy is a blunt-tipped plastic blade originating from the modified 1 ml syringe cap (Figure 1).

Figure 5: Camera otoscopy with modified plastic tip.



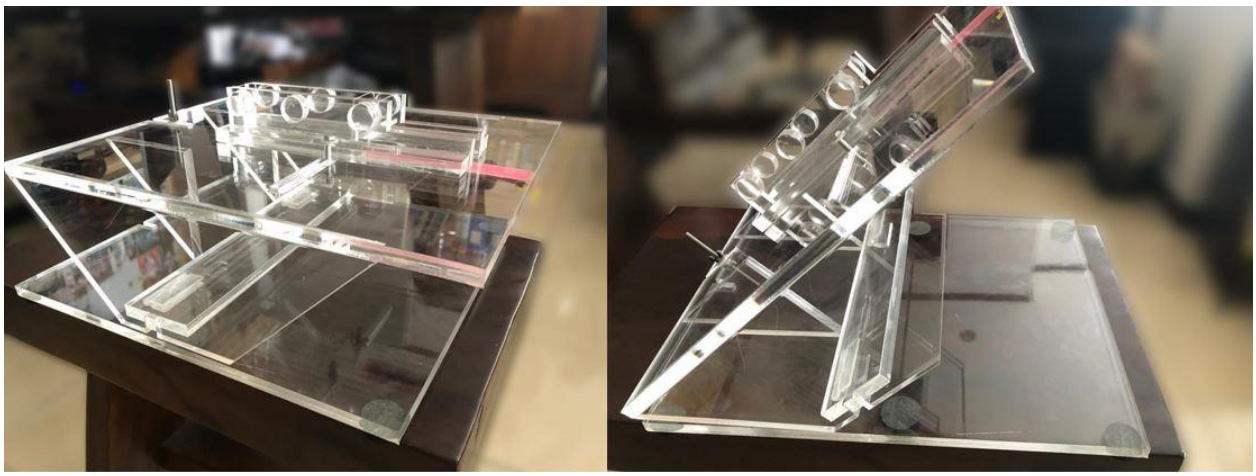
This tip can be made of a syringe cap that is curved using heat, is smoothed, and curved at the tip to prevent injury to the rat's airway. The power source in this otoscopy is obtained from a mobile phone which also functions as a screen to display images. As a tool to reveal the tongue, a cotton swab was used to roll the rat's tongue outwards. The endotracheal tube used was a 16 G intravenous catheter which was directed using an introducer made of blunt-tipped wire (Figure 2). The introducer uses a blunt-tipped wire which is glued to a 1 ml syringe for ease of use.

Figure 6: The endotracheal tube uses a 16 G intravenous catheter

Intubation procedure

The rats were anesthetized with Ketamine (50 mg/kg body weight) and Xylazine (5 mg/kg body weight) by intramuscular injection into the thigh muscle. After the rats were sedated and limp, an ECG monitor electrode was placed on the chest and thighs and peripheral oxygen saturation in the tail. The rat is positioned supine dorsally (half lying 30-45^{o}) with the head above. As fixation, the upper incisors of the rats were treated using rubber on an intubation base. A tilt-changeable base is used as the basis for the action of intubation (Figure 3).

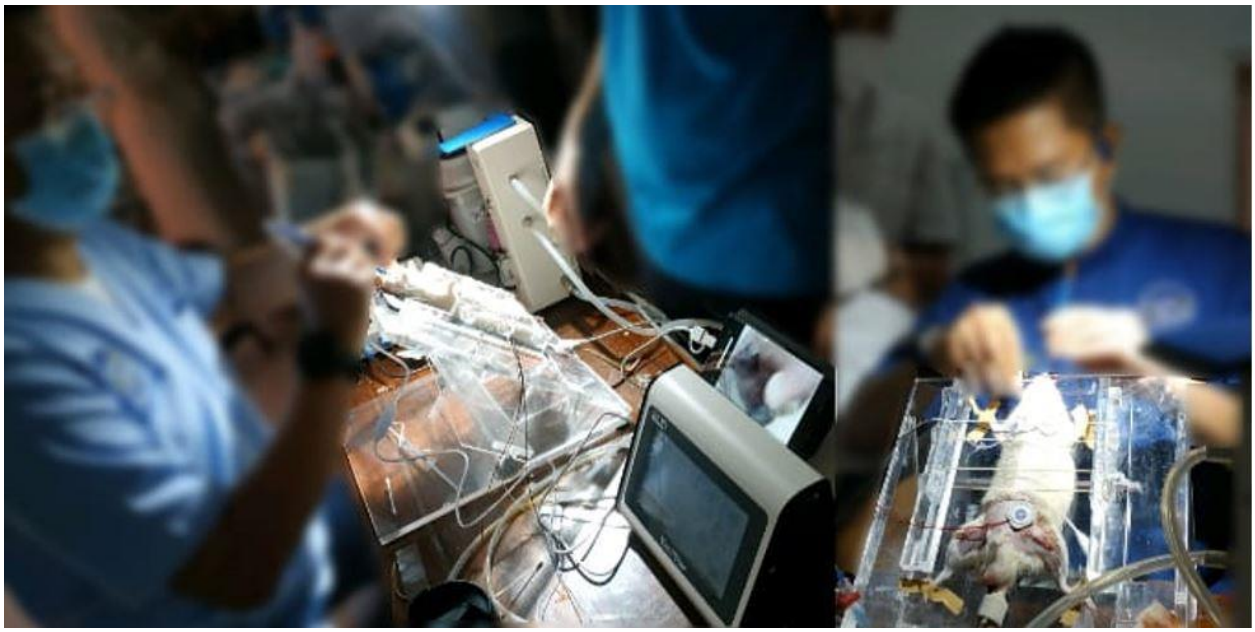
Figure 7: Tilt-adjustable acrylic intubation base



The intubation operator opened the rats' mouth with their right hand and rolled their tongue out using a cotton swab. The operator's left hand enters the intubation device. The airway is visualized on a telephone screen respectively the cavity oropharynx, then the larynx of the vocal cords. The vocal cords were moistened with 2% lidocaine local anesthetic using the tip of a cotton swab. After the vocal cords are anesthetized by fear of opening the vocal cords, the endotracheal tube (16 G intravenous catheter) is inserted using a conveyor. After the catheter is inserted into the trachea, the insert cannot be removed, and the intubation device is removed

from the oral cavity. Then the endotracheal tube is connected to the oxygen source. The position of the operator when performing the procedure can be seen in Figure 4.

Figure 8: The position of the intubation operator when performing the action. Note that the anvil is tilted 45º during action.



The correct placement of the tube was confirmed by auscultation using a neonatal stethoscope and the movement of the rat's chest was symmetrical and rhythmic. The endotracheal tube was fixed in the mouth of the rats using a rubber band. This study used a small animal ventilator as a source of oxygenation and ventilation. Gaseous anesthesia is given using an isoflurane vaporizer.

RESULTS

In this study, 32 rats were intubated. The intubation procedure can be performed simply and quickly. The time taken for intubation ranges from 1 minute. It takes practice and habituation, two anesthetists who perform the intubation procedure need 2-3 experiments on rats before reaching the ability to intubate the rats smoothly. Fourteen of the 32 intubated rats were alive, awakened from anesthesia and under extubation. There were no complications such as stridor or voice disturbances after this intubation procedure. The other eighteen rats were not extubated alive because this was part of the treatment in another study. Of the 32 rats that were subjected to intubation, 2 rats failed to intubate due to spasm and bradycardia. Both occurred in the rats that were the initial sample and could result from a lack of operator experience and skills.

DISCUSSION

In this study, the authors describe the use of intubation equipment in rats that can be easily obtained and modified. The use of a camera with a light source greatly facilitates visualization of the airway structure of very small rats and avoids damage and trauma to the airway structure. By using this technique, generally the intubation procedure can be performed by one operator, sometimes requiring the assistance of one assistant to fix the tongue or help to get instruments.

There is literature describing the method and use of endoscopic equipment and video laryngoscopy specifically for intubation of small animals but these are generally expensive and difficult to obtain[5–9]. The technique used in our study was previously described in research by

Clary in 2003. Clary uses a technique similar to intubation in humans where the operator is right-handed, the scope is operated using the left hand, while the right hand uses cotton buds and inserts the endotracheal tube (ETT) previously attached to the stylet[10]. The advantage of this method is that it's similar to human intubation techniques, thereby simplifying the learning curve for operators with medical backgrounds. Clary's research uses special video-endoscopy equipment.

The research by Balzer in 2020 used commercially available ear wax removal kits and is very similar to the author's research[11]. The advantage of using this tool is that it does not require special veterinary equipment which can be very expensive and difficult to obtain[12,13]. The blade of ear wax removal is analogous to the blade of Miller's laryngoscope used in humans. The study by Samsamshariat used a Miller laryngoscope size 0 and a small diameter wire as a stylet which is commonly used for intubation in neonates and premature infants[14]. The method by Samsamshariat provides 100% success rate and no serious complications.

Another research used a blind technique, which does not use direct visualization tools for the larynx. The drawbacks of the blind technique require several attempts to successfully position the endotracheal tube in the correct position, putting it at risk of traumatizing the very fine structures of the rat airway[15–17]. Research by Zheng in 2020 compared 3 techniques for intubation in mice, namely blind technique, transillumination and direct incision in the trachea. Zheng's research found that the blind technique outperformed other techniques with shorter intubation times, fewer trials and less complications of rat airway damage than other methods[18].

The foundation used in this study uses modified acrylic so that the rat can be positioned supine, properly and safely fixed so that they do not move and can be tilted so that the intubation action can be more ergonomic for the operator. The tiltable rat intubation platform is made using acrylic and is a modification of the Intubation Platform for Rat (IPR) produced by Penn-Century™. The IPR manufactured by Penn-Century™ is discontinued. This acrylic base has been described in other studies. Research by Rendell in 2014 as part of a thymectomy study in adult rats using an acrylic foundation[19].

In this study, the authors applied topical lidocaine to the larynx and vocal cords. Two percent of Lidocaine was applied to the vocal cords and larynx using the tip of a cotton swab moistened with local anesthetic. In the authors' observations, topical application of 2% lidocaine gave a good effect (vocal cords open, decreased airway reflex, and prevented laryngeal spasm) after waiting for its onset for 60 seconds. There were no side effects or toxicity symptoms in the rats given topical anesthesia. The use of topical anesthetics for intubation in rats was also mentioned in the study by Rivard[2].

The use of plastic and cotton to manipulate the airway of rats in this study aims to reduce the risk of trauma and complications. The tip of the plastic blade in this study was made by modifying a 1 mL syringe needle cap which was split and formed a curved tip and smoothed the edges to minimize trauma due to friction. In the future, the use of 3-dimensional printers to print better blades will be possible to provide more ergonomic equipment and minimize injury to the rat's airway[20].

When intubating rats, one of the factors' influencing visualization is the technique to expose the rat's tongue. We found that using dry cotton swabs to roll the rat's tongue out was the most effective method. The method of exposing the tongue using dry cotton swabs was also mentioned in another study and was said to provide a good visualization of the laryngeal

structure of rats[2,21]. Research by Rendell used tweezers to expose the rat's tongue, but this technique requires assistance and the likelihood of injury to the rat's tongue is bigger[19]. To confirm the placement of ETT in the trachea, the authors used the auscultation technique as in general human intubation. The stethoscope used is a stethoscope for neonates. The author's experience of this technique is quite reliable and found no false routes that were not detected. Breath sounds are heard in both chest fields after the ETT is connected to the ventilator. Several other studies used a drop of water in an infusion tube connected to the ETT, the breath air will move water droplets that would not be found in the event of esophageal intubation[22–24]. This method requires the rat to continue to breathe spontaneously.

The complications that the authors found in this study were spasm and bradycardia. Some of the factors that may influence the occurrence of complications are the operator's ability that has not been well honed, which makes intubation attempts to be done several times and anesthesia that is too shallow, causing spasm in the rat's airway. In the literature, complications accompanying intubation in rat include hypersalivation and plugging (due to excessive saliva production), edema and airway bleeding (due to repeated intubation trials), spasm (due to inadequate anesthesia) and anesthetic drug overdose[2,5,23]. Injury at the microscopic level after oral intubation in mice was described by Jahshan in 2018 who found that epithelial and ciliary damage to the airway was found in all rats. who were intubated primarily on ETT use for more than 6 hours[25].

The main advantages of our method are simplicity and relatively low cost to assemble the required tools. The author's experience in using this tool is also straightforward and only takes a short amount of time to achieve the learning curve needed to master it. The operator's background is an anesthetist who is accustomed to performing intubation in humans, where there are similar structures in the airways of humans and rats so that this eases the learning curve. More training and time may be required for researchers from different professional backgrounds to reach the required level of proficiency.

CONCLUSION

The intubation in mice described in this study uses equipment that is inexpensive and easy to obtain, easy to study and has a high success rate.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

PSA, SS, and NMR have developed the research protocol and performed the experimental activities. ER and PSA reviewed and analysed the experimental findings. PSA, WW and YW proposed the ethical application, drafted the manuscript, and submitted to the journal. All authors contributed and gave comments on the research work.

ETHICAL CLEARANCE

The experimental action and protocol have been approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya Indonesia with the number No. 2.KE.015.02.2020.

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Research Journal of Pharmacy and Technology

Temporary Paper ID: **23123182844789229** Date of Submission: **23-Jan-2023**

Paper Title: **Simple Method Laryngoscope on Rat Intubation**

Authors: **Prananda Surya Airlangga; Nancy Margarita Rehatta; Soetjipto; Eddy Rahardjo; Widjiati; Yopie Wiguna**

Dear Author(s),
Regards,

Thanks for submission of paper at enlisted journals of 'A & V Publications'. It is our great pleasure to inform you that your manusccript titled: **"SIMPLE METHOD LARYNGOSCOPE ON RAT INTUBATION"** has been published in **VOLUME - 16, ISSUE - 10** of **Research Journal of Pharmacy and Technology.** with published paper ID : **2023-16-10-62**.

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