A new video laryngoscope specialized for endotracheal intubation in mice

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Abstract
Background: Mechanical ventilation is indispensable in many animal experiments, and establishing a stable airway to control breathing is critical. However, endotracheal intubation in small rodents is very difficult due to the lack of visibility of the epiglottis. In addition, traditional blind endotracheal intubation methods usually cause laryngopharyngeal injury and can even result in death; thus, a noninvasive endotracheal intubation device is needed. Results: The video laryngoscope required significantly less time and fewer attempts to achieve successful intubation. The incidences of vomiting reflex, asphyxia and injury were significantly lower in video laryngoscope group. In addition, the time elapsed until the first feeding postextubation was less in video laryngoscope group, indicating faster recovery. Conclusions: The new video laryngoscope endotracheal intubation device used in this study is simple, noninvasive, safe and practical.

Background
Small rodents such as rats and mice are used in more than 90% of mammalian experiments in biomedical studies. In many animal experiments that involve imaging[1–4]and long-term surgery[5–9], mechanical ventilation is needed to assist breathing. Establishing a stable airway to control breathing is the prerequisite. However, the anatomy of small rodents (Figure 1A) is quite different from that of humans due to lack of visibility of the epiglottis. Traditional blind intubation and other methods are time consuming and can easily cause laryngopharyngeal injury and even death. Therefore, a new noninvasive device for endotracheal intubation in small rodents is needed to address this problem. In this study, a new device for endotracheal intubation in mice was compared with traditional endotracheal intubation techniques such as blind intubation and transillumination of the neck. And to investigate the safety and practicability of the new video laryngoscope specialized for endotracheal intubation in small rodents.

Results
After extubation, all mice were returned to their respective cages for observation and feeding for one week. Compared with the other two groups, the group V required less time and fewer attempts to achieve successful intubation ($P<0.05$). Mice from group V experienced less vomiting reflex, asphyxia and injury during intubation ($P<0.05$). The time elapsed before first feeding postextubation was
significantly less in group V (P<0.05). However, the 24-hours and one-week survival rates did not reach significant differences (P>0.05) (Table 1).

Discussion
Mice are the most commonly used and smallest mammal species in the laboratory. Mice have been clearly and widely demonstrated to be valuable as models of human disease and to promote our understanding of human biology and disease[10]. Mechanical ventilation is required in many medical biological research studies, such as in studies that involve heart disease models, lung disease models and surgical operation in mice. In addition, endotracheal intubation in mice is necessary for experiments involving intratracheal linstillation of various substances[11], repeated pulmonary functional assessments[10,12] and mechanical ventilation[13]. However, due to the anatomical characteristics of rodents, such as small body, large incisors, tight mandible, small larynx, narrow trachea, rapidly mobile vocal cords and so on[14], tracheal intubation is difficult in operations. Furthermore, incorrectly performed endotracheal intubation may lead to complications associated with intubation such as oral bleeding, pharynx edema, laryngeal perforation and esophageal mispositioning[15] and may cause failure of the experiment or affect experimental outcomes. Therefore, it is important to identify a safe, practical and reliable method of endotracheal intubation for use in mice.

To solve the difficult problem of mechanical ventilation in the laboratory, many researchers have sought to develop methods and tools for endotracheal intubation in rodents. Endotracheal intubation methods and tools in rats have been extensively described in the literature[16–23]. However, due to the large differences in body weight and body length between mice and rats (30 grams vs 300 grams), the difficulty of endotracheal intubation in mice was greatly increased, and many methods of endotracheal intubation in rats are not applicable to mice. Therefore, many studies have reported methods and tools for endotracheal intubation in rats, while methods and tools for intratracheal intubation in mice are rarely reported. An endotracheal intubation method for use in mice was first reported by Ho and Furst in 1973[13,24]. Subsequently, some additional methods of endotracheal intubation in mice were reported[13,14,25–32], although these methods are still characterized by
some problems and are, therefore, not ideal. The main problems are as follows: First, tracheostomy can increase the infection rate and the death rate, preventing its use in long-term experiments, and inflammatory reactions may lead to experimental bias. Second, although some of the above-mentioned methods improve the field of view of endotracheal intubation, the oral space in mice is very small; therefore, when endotracheal intubation is performed, the tracheal catheter blocks the field of view of endotracheal intubation, resulting in blind intubation. Third, some endoscopic methods are nonprofessional endotracheal intubation tools, which require extensive training and are high in cost. In addition, existing video laryngoscopes have many parts and are very large when assembled, making them inconvenient to use and transport.

The new video laryngoscope adopted in this study is simple, noninvasive, safe and practical. Similar to the clinical application of video laryngoscopes, the left hand is used to hold the video laryngoscope, and the right hand is used to hold the tracheal catheter under guidance of the visual field show by the video laryngoscope for endotracheal intubation. According to the analysis of the experimental results (Table 1), the practice ability and safety indicators of mice in group V were significantly different from those of the other two groups. The differences between group B and group T did not achieve significance. According to the comprehensive comparison, the new video laryngoscope used in this study can be applied to endotracheal intubation in mice, which is superior to traditional methods. The device not only can improve the success rate of endotracheal intubation but also reduce damage to the larynx during endotracheal intubation, and it is easy to operate and transport. After practice, only 20 seconds are needed to perform endotracheal intubation using the device.

The limitations of this study are as follows: first, the sample size of each group is small and some of the observational indicators are subjective; however, observational studies are susceptible to measurement and selection bias[33]. Second, there was no gender statistical comparison of endotracheal intubation methods in mice. Third, the subjects were only mice, and endotracheal intubation was not studied in larger rodents, such as rats. In the following study, we will study endotracheal intubation methods in larger rodents.

Conclusions
The new video laryngoscope tracheal intubation device used in this study can improve the success rate of tracheal intubation in mice, easy to carry, reduce injury and death caused by intubation. It is simple, noninvasive, safe and practical to use in tracheal intubation in mice.

Methods
This study was approved by the expert committee of medical ethics review of Wannan Medical College (Wuhu, Anhui, China) and were carried out in accordance with the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europe.

Animals
Thirty C57BL/6J mice (25–30 grams) were provided by the experimental animal center of Wannan Medical College. These mice were randomly divided into the following three groups containing 10 mice each: a blind intubation group (group B), a transillumination of the neck group (group T) and a video laryngoscope group (group V).

Tracheal Catheter:
22 gauge passivated intravenous needles were used as tracheal catheters in this study. The length of the needle was slightly shorter than the puncture needle sleeve, and the curve was approximately 15 degrees.

The Video Laryngoscope
The video laryngoscope (Figure 2 A, B) consists of two main parts: a display screen and a probe. The probe was specifically designed to adapt to the oral anatomy of small rodents. In addition, an secure digital memory card was built into the device to store photographs during intubation.

Anesthesia and Preparation
Mice were anesthetized via intraperitoneally injection with 3% pentobarbital sodium (0.05 g/kg) and atropine (1 mg/kg). After disappearance of the righting reflex, the mice were fixed in a supine position on custom-made tracheal intubation operating platform with a gauze roll placed behind the back of the neck for support. The upper incisors of the mice were pulled back and fixed with a surgical silk thread. (Figure 3 A, B). It should be emphasized that postural position is very important for endotracheal intubation. This position facilitates endotracheal intubation (Figure 1 B). Three different
methods of endotracheal intubation were tested. After completion of endotracheal intubation, the vital signs of the mice were monitored. The mice were returned to their cages for further observation following extubation. Two weeks after observation following extubation, the mice were anesthetized using 2.5% isoflurane, and the best effort was employed to minimize the pain, and sacrificed by exsanguination.

*Three Methods of Endotracheal Intubation*

*Group B:* After fixing the mouse’s position (Figure 3, A, B), the tongue was pulled out of the mouth using forceps with the left hand, and secretions in the mouth and throat were cleaned with cotton swabs. A tracheal catheter was gently pressed upward against the tongue and slightly slid down according to the breathing tendency. When the tracheal catheter was inserted into the trachea, specific friction can be felt due to the discontinuous contact with the tracheal ring. In addition, circular fog spots should be observed on the glass slide; otherwise, the catheter should be immediately removed and reinserted.

*Group T:* After opening the mouth and cleaning the secretion, as described above, an assistant placed a strong cold light source near the neck of the mouse. The opening and closing of mouse’s glottis can be seen when the strong light penetrates through the thin skin and the muscle layer of the neck. A tracheal catheter was gently pressed upward against the mouse’s tongue and inserted swiftly into the trachea at the moment when the glottis was opened. The method used to verify successful intubation is described above.

*Group V:* After opening the mouth and cleaning the secretion, as described above, the video laryngoscope was placed in the mouse. The front of the probe was gently pressed against the tongue and slid slightly downward. The laryngoscope was slowly moved to search for the epiglottis on the video display. When the epiglottis was found, the probe was advanced forward to expose the glottis beneath the epiglottis. Under the guidance of the video laryngoscope, a tracheal catheter was inserted into the trachea at the moment when the glottis opened (Figure 2C, D).

*Observation Indicators*

The following practical indicators were evaluated: Time consumed for intubation, number of attempts
before successful intubation, one-time success rate of intubation.

The following safety indicators were evaluated: Incidences of vomiting reflex, asphyxia, and injury; elapsed time before first feeding postextubation; 24-hour survival rate; and one-week survival rate.

**Statistical Analysis**

SPSS 16.0 (SPSS Inc., Chicago, IL, USA) statistical software was used to analyze the data. The measurement data were expressed as the means ± standard deviations. One-way Analysis of Variance was used for comparisons among groups, and Fisher’s exact probability method was used for post hoc analysis. The $P$ less than 0.05 ($P<0.05$) was considered significant difference.

**Abbreviations**

Group B: the blind intubation group

Group T: the transillumination of the neck group

Group V: the video laryngoscope group.

**Declarations**

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**Availability of data and materials:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Authors’ contributions:**

GQS helped design the study, analyze the data, prepare all figures, and write the manuscript. WJG helped design the study, analyze the data, and write the manuscript. QX helped analyze the data, conduct most of the experiments, and review the manuscript. CL helped with animal care and conduct some of the experiments. SPF helped design the study, analyze the data, and write the manuscript. All authors read and approved the final manuscript.

**Competing interests:** The authors declare that they have no competing interests.

**Consent for publication:** Not applicable.

**Ethics approval and consent to participate:**
All animal procedures and experimental protocols were approved by the expert committee of medical ethics review of Wannan Medical College (Wuhu, Anhui, China) and were carried out in accordance with the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europ.

Authors’ information

GQS approves the final manuscript, attests to the integrity of the original data and the analyses reported in this manuscript, is the archival author, and is accountable for all aspects of the work. WJG approves the final manuscript and attests to the integrity of the original data and the analyses reported in this manuscript.

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Tables

Table 1 The results of endotracheal intubation in three groups of mice were compared (x±s, n=10/group)

| Index                                | Group B         | Group T         | Group V         |
|--------------------------------------|-----------------|-----------------|-----------------|
| Time required for intubation (s)      | 103.6±53.35     | 74.8±37.53      | 24.2±5.94★      |
| Attempts before successful intubation | 3.5±1.84        | 2.6±1.26        | 1±0★           |
| One-time success rate of intubation (%) | 20±2/10        | 30 (3/10)       | 100 (10/10)★    |
| Incidence of vomiting reflex          | 2.3±1.83        | 1.4±1.07        | 0±0★           |
| Incidence of asphyxia                 | 1.9±1.73        | 1.2±1.14        | 0±0★           |
| Incidence of injury of intubation (%) | 70 (7/10)       | 60 (6/10)       | 0★              |
| Time before first feeding postextubation (h) | 20.89±7.06   | 18±5.44         | 10.9±1.20★      |
| 24-hr survival rate after extubation (%) | 90 (9/10)      | 100 (10/10)     | 100 (10/10)     |
| One-week survival rate after extubation (%) | 90 (9/10)      | 100 (10/10)     | 100 (10/10)     |

Note: ★P<0.05, data are presented as the means ± standard deviations. n = 10/group.

Group B: the blind intubation group Group T: the transillumination of the neck group Group V: the video laryngoscope group.

Figures
Figure 1

The schematic diagram. A. the schematic diagram of oropharyngeal anatomy during endotracheal intubation in mice. B. the position in which the oral and pharyngeal axes almost overlap during endotracheal intubation in mice.

Figure 2

The video laryngoscope and endotracheal intubation in mice. A. and B. show the video laryngoscope. C. and D. show views of the epiglottis of mice during intubation acquired by the video laryngoscope.
Figure 3

The custom-made tracheal intubation operating platform. A. and B. show the custom-made tracheal intubation operating platform and position required for endotracheal intubation.

Supplementary Files

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