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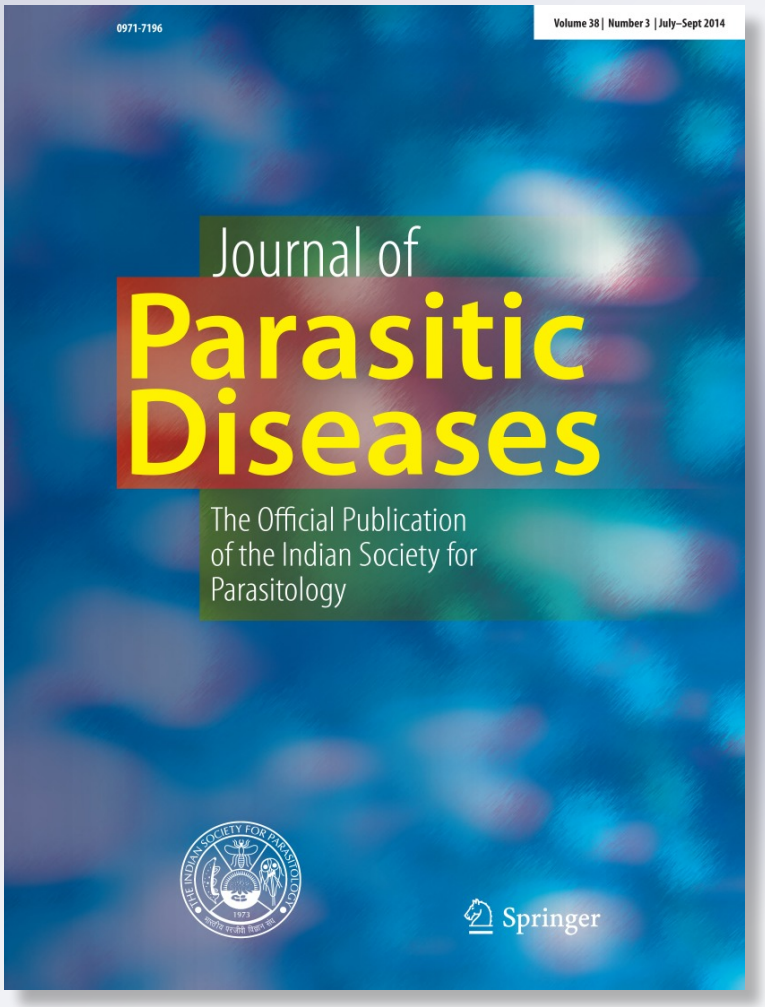

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ORIGINAL ARTICLE

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Comparison of two methods of *Marshallagia marshalli* donor sheep production

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Abstract *Marshallagia marshalli* is found in the abomasums of sheep, goats and wild ruminants in tropical and sub tropical climates. In Iran among different species of Ostertagiinae that can infect sheep, *M. marshalli* is currently the major cause of parasitic gastroenteritis in ruminant. Having a donor animal is essential for various studies. In the present study we compared the efficacy of two different method of *M. marshalli* donor sheep production. In the first method, *M. marshalli* donor sheep was produced by transplanting of adult forms of this worm into abomasum of a lamb (6 months of age) through a surgically established cannula. In the second method, 5,000 infective larvae (L3) from the female *M. marshalli* culture were given to a worm-free lamb of 6 months age through a stomach tube. After 3 days of transplanting, few eggs of *M. marshalli* appeared in the cannulated lamb's faeces. The number of eggs per gram of faeces (EPG) increased in the following days. The average number of EPG reached up to 23.5 ± 11.26 per day in 2 months. In larval infected lamb by day 21 post infection the eggs were appeared in faeces. The average number of EPG reached up 53.5 ± 42.5 per day in 2 months. In comparison between cannulation and larval infected, the number of eggs laid by worms transplanted in cannulated lamb was less than that of larval infected lamb. However, the abomasal cannulation method seems more preferable due to some advantages such as defined number of worms transplanted into abomasums, rapid access to the eggs and their culture.

Keywords *Marshallagia marshalli* · Cannulation · Donor sheep · Stomach tube

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Introduction

Species of Marshallagia (Orloff 1933) are typical abomasal parasites in free-ranging and domesticated ungulates in temperate climatic zones throughout the world (Soulsby 1982, Hoberg et al. 2001). In the global fauna of the Ostertagiinae, there is 13 genera including those characterized by a bursal formula of 2–2–1 (Teladorsagia) and those in which the lateral rays describe a 2–1–2 pattern (Marshallagia, Ostertagia) (Lichtenfels et al. 1988). Ostertagiinae has a direct life cycle which after ingestion of L3, larvae exsheaths in the rumen and further development takes place in the lumen of an abomasal gland. Two parasitic moults occur before the L5 emerges from the gland around 18 days after infection in order to become sexually mature on the mucosal surface (Taylor et al. 2007). Prevalence of this nematode has been reported ranging from 0.72 to 84.1 % in domestic animal from various parts of the world (Anderson 2000, Tariq et al. 2008, Khan et al. 2010, Khalafalla et al. 2010, Borji et al. 2011). In Iran among different species of Ostertagiinae that can infect sheep, *M. marshalli* is currently the major cause of parasitic gastroenteritis in ruminant (Eslami et al. 1979). The available information about *M. marshalli* in the world is morphological characterization based on scanning electron microscopy and DNA evidence (Dallas et al. 2001, Borji et al. 2011). The *M. marshalli* infection is generally mixed

with other gastrointestinal nematodes. For pathological and immunological studies having a donor animal is needed. Although many studies have been carried out with donor sheep (Scott and Mckellar 1988), still there is no evidence of producing *M. marshalli* donor sheep in the world. In this study *M. marshalli* donor sheep production was compared by transplanting of adult form of this worm into abomasum of the lamb through a surgically established cannula and transferring of infected larvae to abomasum of the lamb through a stomach tube.

Materials and methods

Animals

Two sheep, 6 months old male Baluchi race that were born and reared under conditions which minimized the risk of parasitic infection were used in this study. These sheep were housed individually and provided with water ad libitum. They were treated with broad spectrum anthelmintic (Albendazol 7.5 mg/kg oral dose). Rectal faecal samples were examined for parasitic stage before the beginning of the experiment.

Cannulation method (nematode collected and identification)

For producing donor sheep via cannulation, abomasal cannula was surgically implanted in the 6 months old sheep under local anesthesia. Lamb was placed in left lateral recumbency and right paracostal region was prepared for aseptic surgery. A 10 cm long skin incision was made 5 cm caudal to the last rib. The abomasum was exteriorized and isolated with a moistened surgical towel. Stab incision was made at the right abdominal wall caudal to the last rib and a Foley catheter (18–30 French) was inserted through the stab-incision into the abdominal cavity. A purse-string suture (2 cm in diameter) using 2-0 polyglactin 910 (Vicryl; Ethicon) was placed in the abomasal wall, midway between the greater and lesser curvature in the pyloric antrum. A small stab incision was made at the center of preplaced purse-string suture, and Foley catheter tip inserted into the abomasal lumen. The bulb of the Foley catheter was inflated with saline and purse-string suture was secured around the tube. Three absorbable sutures were preplaced between the pyloric antrum and the body wall where the tube was exited. The stomach was drawn to the body wall by placing traction on the catheter and the preplaced sutures were tied. Exteriorized tube was sutured to the skin of the right abdominal region with a Roman sandal suture pattern. The laparotomy incision was closed routinely and bandage was placed around the sheep's abdomen and over the tube.

For implanting an appropriate number of adult worms into abomasum, adult nematodes were collected from the abomasums of sheep which carried out from slaughterhouse to parasitology lab. Speciation was done using light microscopy and based on standard identification keys to keys (Yamaguti 1961).

Five thousands adult form of this worm were transplanted into abomasum of the lamb through cannula (3,600 female worms, 1,400 male worms).

Stomach tube method

For producing donor sheep via stomach tube, the appropriate numbers of infective larvae were required. In order to preparing adult female of *M. marshalli*, the abomasums of sheep were carried from slaughterhouse to parasitology lab. Female of *M. marshalli* were fragmentized and eggs were removed from the worm's body. *M. marshalli* eggs are ellipsoidal and much larger than other nematode species (>150 μm). The collected eggs were incubated at 27 °C and after a week the infected larvae were collected and counted. By this time they were fed with *E. coli*. Five thousands infective larvae were transplanting into rumen of the lamb through stomach tube.

After inducing infection via stomach tube and cannula, two lambs were housed indoors on concrete floors covered with clean straw. The faecal samples were taken daily post infection and were transferred immediately to parasitology lab in order to estimate the egg count. The egg per gram of faeces (EPG) measurement was conducted by Clayton lane method by using the saturated salt solution.

Results

In cannulated sheep, initial appearance of *M. marshalli* egg in faecal samples was occurred by day 3 post infection. The egg counts were two. The EPG increased in following day which maximum number of eggs per gram of faeces on day 28 post infection was 47. In some days, the EPG was become lower than previous day but EPG increased again in next days. The average number of EPG reached up to 23.5 ± 11.26 per day during 2 months.

In infected sheep by stomach tube, the eggs were appeared in faeces by day 21 post infection. The Egg counts were three. The average number of EPG reached up 53.5 ± 42.5 per day in 2 months (Fig 1).

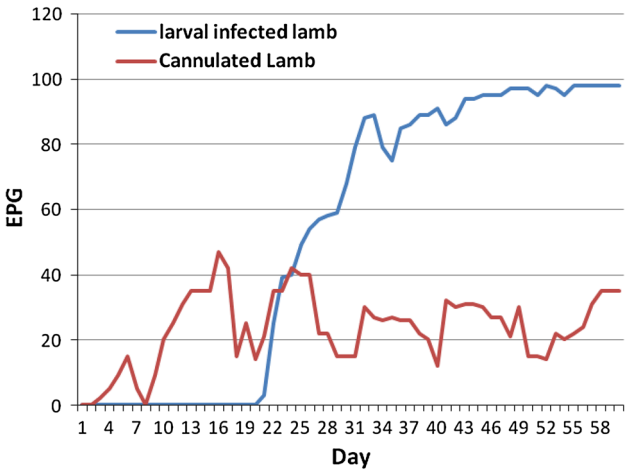

Fig. 1 Faecal egg count in two lambs infected with *Marshallagia marshalli* via stomach tube and cannulation

Discussion

According to our results, we could transfer certain numbers of male and female of *M. marshalli* to abomasum of a donor sheep by cannulation method for the first time in the world. With cannulation method, eggs of *M. marshalli* were appeared in faecal sample of infected sheep after 2 or 3 days. Feeding a lamb with infected larvae via stomach tube seems to be an easier technique. Moreover, the numbers of adult worms, which will be developed after infecting lamb with L3, was not apparent and egg worm will be appeared in faecal samples 3–4 weeks after of feeding time but EPG increasing faster than previous method.

The advantages of employing the cannulation technique on the basis of our findings include: quick access to abomasum in order to transfer adult worm, to have a chance of increasing the number of worms in abomasum over different stages when the number of eggs in faecal is small, no need to wait for a long time for worm maturation, quick access to the egg worm in faeces in order to generate more worms. The disadvantages of the manipulating the cannulation technique are as follows: A need for surgery and a cannulation skill, a need for providing an appropriate cannulation, intensive cares after surgery, the possibility of the old adult worm with low chance of fertility being transferred to the abomasums. The advantages of feeding infected larvae via stomach tube are as follows: An easy technique, no need for special cares and concerns after feeding, the generated adult worms have a great chance of fertility which will lead to be producing more eggs.

The disadvantages of feeding infected larvae via stomach tube are as follows: Due to low fertility of *M. marshalli*, producing enough larvae from culturing is time consuming, a great number of infected larvae are lost while they are transferred through stomach tube, it takes much time to generate egg worm in faeces.

The cannulation method has been manipulated for other worms as well. For instance, cannulated lambs were used for measurement of pathophysiological evaluation in *Teladorsagia circumcincta* (Simpson et al. 2009).Waller et al. (1994) has employed cannulation method in order to study the special effects of nematophaogus fungi to control the free living stage of nematode parasite of sheep. Also, Pfeffer et al. (1996) for review of sequetal cellular and humoral responses in the abomasal mucosa and blood of Romney sheep dosed with *Trichostrongylus axei* has transfer Trichostrongylus through cannula in lamb. Alborzi et al. (2007) another scientist in Iran has used cannulation method for producing *Ostertagia circumcincta* donor sheep.

In conclusion, cannulation method is recommended for considering the drug efficacy in farm condition, to investigate herbal drug and evaluate immune response against adult worm.Besides, larval infection through stomach tube is proposed for pathophysiologic study and producing large number of infected larvae .

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