An Epidemiological Study of Hypoderma Infection and Control Using Ivermectin in Yaks in Qinghai Province, China

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ABSTRACT. The prevalence of Hypoderma spp. in yaks grazed in the east of Qinghai province was investigated in 2008. In this area, the prevalence in young yaks (1- to 3-year-old) was very high at 82.2–98.7%, whilst in adult yaks (4-year-old and older), the prevalence was 42.4–50.6%. The seasonal development and migration pattern of Hypoderma larvae in yak bodies was found to be similar for different locations in this area. The numbers of first, second and third instar larvae detected in yak bodies peaked in October, December and March, respectively. Different doses of ivermectin (125 to 500 µg/kg body weight) almost completely dewormed the larvae from yaks, suggesting that using a quarter of the prescribed dose (500 µg/kg body weight) was effective. In October of each year between 2009 and 2012, ivermectin (125 µg/kg body weight) was administered to a total of 562,995 yaks grazed in four counties in Qinghai province, and the prevalence of Hypoderma larval infection in yaks was reduced to 0.5–1.0%.

KEYWORDS. China, Hypoderma, ivermectin, Qinghai, yak.

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Qinghai province is located in the northeast Qinghai-Tibetan plateau in west China. Since the average elevation is higher than 3,000 meters, nomadic grazing of high-altitude adaptive animals, such as yak and Tibetan sheep, is very popular. In this province, more than 4.8 million yaks are grazed at an elevation of 3,000 to 4,500 m.

Warble flies (Hypoderma spp.) are commonly found in yaks grazed in the province. Hypodermodosis is a serious disease affecting livestock throughout the world, and two species of Hypoderma, H. bovis and H. lineatum, mainly affect cattle [10]. However, in yaks, a third species, H. sinense, is also commonly found [12, 15]. The overall prevalence of Hypoderma larval infection in yak is around 70–95%, reaching 100% in some areas [24]. Hypodermodosis has also been found in cattle, wild animals, such as pikas [23] and chirus (Tibetan antelopes) [6, 8, 17], and occasionally in sheep, deer and horses in Qinghai province [24]. Moreover, infection in humans, especially in nomads who have regular contact with yaks, has been frequently recognized [24]. We previously conducted a preliminary control study in Qinghai province to compare the effect of ivermectin administration (500 µg/kg body weight) to yaks in different months and found that treatment of yaks in October was most effective [13].

Hypodermodosis is a subcutaneous myiasis which causes significant economic losses in the animal production industry [2, 4, 10] due to reduced weight gain, milk production and leather quality [24]. Even though the economic and zoonotic problems of warble flies are quite significant, systematic control measures against the parasite infection have not been used in Qinghai province. To establish a potential control scheme, we investigated the seasonal migration pattern of Hypoderma larvae in yaks in different locations in the province and conducted a large-scale anthelmintic control program targeting the first stage larvae in four counties (Maqin, Yushu, Guinan and Haiyan counties) in Qinghai province between 2008 and 2012.

MATERIALS AND METHODS

Study sites: The study sites were Yushu county at Yushu state and Maqin county at Guoluo state located in southeast Qinghai; Guinan county at Hainan state in middle-east Qinghai; and Haiyan county at Haibei state in Qinghai Lake area (Fig. 1).

Investigation of the prevalence of hypodermodosis in yaks: The investigation was carried out in Yushu, Maqin and Haiyan counties between March and May in 2008. The skin surfaces of the neck, shoulder, chest and back of 25,769 live yaks were examined for Hypoderma lumps by hand
The lumps were counted, and the prevalence of Hypoderma larval infection and the mean number of lumps in different age groups (1-, 2- and 3-year-old and adult (≥4-year-old)) of yaks were evaluated for each county.

Investigation of seasonal pattern of Hypoderma larval development and migration in yak bodies: The investigation was performed in two counties. One was Yushu county with an average elevation of 4,000 m, and the other was Haiyan county with an average elevation of 3,000 m. Every month from April 2008 to March 2009, five one-year-old yaks were selected from the herd grazed in the counties and autopsied to check for Hypoderma larvae in visceral organs, such as esophagus, rumen, jejunum, colon, diaphragm, spinal canal and in the skin of back. The larvae collected were fixed in 10% formalin and counted, and their instar larval stage was determined as previously described [10, 15].

Determination of an effective dose of ivermectin for Hypoderma larvae: In Yushu, Maqin and Haiyan counties, different doses of ivermectin (Beijing Zhongnong Huawei Pharmaceutical Co., Ltd., Beijing, China) were administered to yaks younger than 2-year-old in October 2008. A liquid ivermectin formula was put into a syringe and splashed onto the yaks back. The yaks were divided into four groups (1,000–1,500 head/group in each county); the full dose group was given a single treatment with 500 µg/kg body weight (bw) which is the dose recommended for the treatment of Hypoderma larvae by the commercial suppliers; the half dose group was given 250 µg/kg bw, the quarter dose group was given 125 µg/kg bw and the control group remained untreated. Between February and April 2009, the yak’s skin surface was hand-palpated to check and count Hypoderma lumps. The neck, shoulder, chest and back were examined. The proportion of yaks used for this study was about 2 to 5% of the total population of the grazed yak herd in each study site.

Statistics: Ninety-five percent confidence intervals (95% CI) of prevalence were calculated on the basis of binomial distributions using the software program R [19].

RESULTS

Prevalence of hypodermosis in yaks: The prevalence in different age groups of yaks in Yushu, Maqin and Haiyan counties is shown in Table 1. All counties showed a similar pattern of age difference in prevalence. In 1-, 2- and 3-year-old yaks was significantly lower (42 to 51%). The number of Hypoderma lumps detected per yak was also higher in young (1- to 3-year-old) yaks than that in adult yaks. It is recognized that the prevalence and the mean number of lumps were relatively higher in Haiyan county than other counties.

Seasonal pattern of Hypoderma larval development and migration in the yak body: The number of larvae and their instar larval stages in the yaks at Yushu and Haiyan counties in each month from April 2008 to March 2009 are shown in Table 2. A quite similar pattern was observed in both
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First instar larvae appeared in the yak bodies in mid-August 2008 and were continually found until February 2009. The number of 1st instar larvae detected per yak was highest in October 2008 and then decreased gradually. The migration pattern of 1st instar larvae in yak bodies was quite similar in the two counties, and thus, the data are combined and shown in Table 3. The majority of larvae were found in the subserosa of the esophagus and rumen. However, although restricted in August and September, parts of the larvae were also found in the subserosa of the small intestine and colon and in the diaphragm and spinal canal.

In November 2008, 2nd instar larvae first appeared on the

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**Table 1. Prevalence of *Hypoderma* larval infection in yaks of different ages in three counties of Qinghai**

| County | Age group | No. examined | No. infected | Prevalence (%) (95% CI) | Mean No. lumps<sup>b</sup> |
|--------|-----------|--------------|--------------|--------------------------|---------------------------|
| Yushu  | 1-year-old| 1,978        | 1,902        | 96.2 (95.2–96.9)         | 13.5                      |
|        | 2-year-old| 2,246        | 2,124        | 94.6 (93.6–95.4)         | 11.2                      |
|        | 3-year-old| 2,580        | 2,156        | 83.6 (82.1–84.9)         | 10.9                      |
|        | Adult     | 3,264        | 1,430        | 43.8 (42.1–45.5)         | 9.8                       |
|        | Total     | 10,068       | 7,612        | 75.6 (74.8–76.4)         | 11.4                      |
| Maqin  | 1-year-old| 2,007        | 1,911        | 95.2 (94.2–96.1)         | 12.4                      |
|        | 2-year-old| 2,151        | 2,023        | 94.0 (93.3–95.0)         | 10.2                      |
|        | 3-year-old| 2,080        | 1,710        | 82.2 (80.5–83.8)         | 11.4                      |
|        | Adult     | 2,667        | 1,130        | 42.4 (40.5–44.3)         | 8.2                       |
|        | Total     | 8,905        | 6,774        | 76.1 (75.2–76.9)         | 10.8                      |
| Haiyan | 1-year-old| 2,340        | 2,250        | 96.2 (95.3–96.9)         | 14.4                      |
|        | 2-year-old| 1,880        | 1,855        | 98.7 (98.0–99.1)         | 15.5                      |
|        | 3-year-old| 1,553        | 1,386        | 89.2 (87.6–90.7)         | 15.2                      |
|        | Adult     | 1,023        | 518          | 50.6 (47.6–53.7)         | 9.8                       |
|        | Total     | 6,796        | 6,009        | 88.4 (87.6–89.2)         | 14.5                      |

<sup>a</sup> 95% confidence interval. <sup>b</sup>Mean number of *Hypoderma* lumps found per yak.

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**Table 2. Monthly change in the mean number of 1st, 2nd and 3rd instar *Hypoderma* larvae found in yaks in two counties of Qinghai between April 2008 and March 2009**

| Year | Month | Yushu county | Haiyan county |
|------|-------|--------------|---------------|
|      |       | 1st instar   | 2nd instar   | 3rd instar   | 1st instar | 2nd instar | 3rd instar |
| 2008 | Apr   | 0            | 0            | 45.8         | 0          | 0          | 46.6       |
|      | May   | 0            | 0            | 30.2         | 0          | 0          | 32.8       |
|      | June  | 0            | 0            | 2.2          | 0          | 0          | 3.4        |
|      | July  | 0            | 0            | 0            | 0          | 0          | 0          |
|      | Aug   | 26.2         | 0            | 0            | 25.0       | 0          | 0          |
|      | Sep   | 40.2         | 0            | 0            | 38.8       | 0          | 0          |
|      | Oct   | 116.0        | 0            | 0            | 102.4      | 0          | 0          |
|      | Nov   | 57.8         | 24.2         | 0            | 58.8       | 37.6       | 0          |
|      | Dec   | 17.6         | 63.2         | 0            | 17.2       | 65.4       | 0          |
| 2009 | Jan   | 12.2         | 75           | 17.8         | 10.2       | 69.0       | 16.6       |
|      | Feb   | 2.8          | 37.8         | 46.2         | 3.6        | 39.8       | 51.6       |
|      | Mar   | 0            | 0            | 66.6         | 0          | 0          | 65.0       |

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**Table 3. The mean number of 1st instar *Hypoderma* larvae detected in various organs of yaks in Yushu and Haiyan counties of Qinghai between August 2008 and February 2009**

| Year | Month | Esophagus | Rumen | Small intestine | Colon | Diaphragm | Spinal canal |
|------|-------|-----------|-------|-----------------|-------|-----------|-------------|
| 2008 | Aug   | 8.8       | 7.6   | 4.6             | 3.2   | 0.5       | 0.9         |
|      | Sep   | 17.1      | 16.3  | 5.0             | 0.8   | 0.5       | 0.3         |
|      | Oct   | 56.2      | 53.0  | 0.8             | 0.8   | 0.8       | 0.8         |
|      | Nov   | 31.5      | 26.8  | 0.8             | 0.8   | 0.8       | 0.8         |
| 2009 | Jan   | 9.9       | 7.5   | 0.8             | 0.8   | 0.8       | 0.8         |
|      | Feb   | 7.3       | 3.9   | 0.8             | 0.8   | 0.8       | 0.8         |
back of animal in both counties. The number of 2nd instar larvae detected per yak increased in December 2008, peaked in January 2009 and disappeared by March. All of the larvae were found in the subcutaneous tissue of the back (Table 2).

In January 2009, 3rd instar larvae appeared on the back of animals, and the number detected per yak peaked in March 2009. Since 3rd instar larvae were found from April to June 2008, it is suspected that they parasitize yaks between January and June (Table 2).

Third instar larvae could easily be distinguished from 2nd instar larvae by the body size and color. The 2nd instar larvae were 11–15 mm long and 3–6 mm wide, while 3rd instar larvae were 23–30 mm long and 12–15 mm wide. The body color of 2nd instar larvae was yellowish white, while that of 3rd instar larvae was yellowish to dark brown.

An effective dose of ivermectin for Hypoderma larvae: The effect of different doses of ivermectin administration on Hypoderma larval infection in yaks is shown in Table 4. The trials conducted in all three counties (Yushu, Maqin and Haiyan) showed similar results. The prevalence in the control groups was very high (93.2–94.6%), and the mean number of lumps found was around 13. In contrast, no Hypoderma lumps were found in the groups treated with 500 and 250 µg/kg ivermectin, and hence, no infected animals were found. The prevalence in the groups treated with 125 µg/kg ivermectin was also extremely low (0.1–0.2%), and the mean number of lumps found was 1.0 to 1.5.

A successful control of wable fly larval infection: Cumulative data from the trial conducted over three years in four counties are shown in Table 5. There was no obvious difference in the annual data between 2009 and 2011 in the four counties. Some yaks remained infected in all counties and in all age groups, but the overall prevalence was <1.0% and the mean number of lumps found was below 1.5 at 5 to 7 months.

**Table 4.** The effect of administration of different doses of ivermectin on Hypoderma larval infection in yaks

| County | Dose (µg/kg) | No. treated | No. infected | Prevalence (%) | Mean No. lumps* |
|--------|--------------|-------------|--------------|----------------|----------------|
| Yushu  | 500          | 1,100       | 0            | 0              | 0              |
|        | 250          | 1,058       | 0            | 0              | 0              |
|        | 125          | 1,350       | 2            | 0.2            | 1.5            |
|        | Control      | 1,064       | 992          | 93.2           | 12.9           |
| Maqin  | 500          | 1,500       | 0            | 0              | 0              |
|        | 250          | 1,258       | 0            | 0              | 0              |
|        | 125          | 1,050       | 1            | 0.1            | 1.0            |
|        | Control      | 1,164       | 1,092        | 93.2           | 12.6           |
| Haiyan | 500          | 1,800       | 0            | 0              | 0              |
|        | 250          | 1,558       | 0            | 0              | 0              |
|        | 125          | 1,250       | 2            | 0.2            | 1.0            |
|        | Control      | 1,364       | 1,290        | 94.6           | 13.7           |

*Mean number of Hypoderma lumps found per yak.

**Table 5.** Prevalence of Hypoderma larval infection and mean number of lumps found in different age groups of yaks after administration of ivermectin in four counties in Qinghai

| County | Age group | No. treated | No. examined | No. infected | Prevalence (%) | Mean No. lumps* |
|--------|-----------|-------------|--------------|--------------|----------------|----------------|
| Yushu  | 1-year-old| 38,050      | 3,797        | 34           | 0.9            | 1.2            |
|        | 2-year-old| 37,542      | 3,916        | 35           | 0.9            | 1.1            |
|        | 3-year-old| 32,039      | 3,114        | 27           | 0.9            | 1.0            |
|        | Adult     | 25,873      | 2,561        | 22           | 0.9            | 1.0            |
| Maqin  | 1-year-old| 44,644      | 4,576        | 45           | 1.0            | 1.1            |
|        | 2-year-old| 40,021      | 4,083        | 35           | 0.9            | 1.4            |
|        | 3-year-old| 31,943      | 3,306        | 24           | 0.7            | 1.1            |
|        | Adult     | 24,434      | 2,592        | 14           | 0.5            | 1.1            |
| Haiyan | 1-year-old| 42,316      | 4,483        | 37           | 0.8            | 1.1            |
|        | 2-year-old| 40,034      | 4,206        | 35           | 0.8            | 1.0            |
|        | 3-year-old| 30,618      | 3,158        | 19           | 0.6            | 1.0            |
|        | Adult     | 23,463      | 2,486        | 13           | 0.5            | 1.0            |
| Guinan | 1-year-old| 51,722      | 5,282        | 43           | 0.8            | 1.2            |
|        | 2-year-old| 43,720      | 4,535        | 37           | 0.8            | 1.2            |
|        | 3-year-old| 31,934      | 3,266        | 24           | 0.7            | 1.4            |
|        | Adult     | 24,642      | 2,612        | 18           | 0.7            | 1.0            |

*Mean number of Hypoderma lumps found per yak.
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post-administration of ivermectin. There was no obvious difference among the data obtained from different age groups or different counties.

DISCUSSION

This survey was conducted in four counties located in the middle-east Qinghai to Qinghai lake area (Haiyan county and Guinan county) and southeast Qinghai area (Yushu county and Maqin county). These two areas are 1,000 km apart with average elevation of 3,000 and 4,000 m, respectively. They have different ecological environments with annual fluctuations in mean atmospheric pressure, temperature and precipitation that could affect the distribution of Hypoderma. However, we did not find any obvious differences in the prevalence of Hypoderma in yaks from these two areas. The results indicate that Hypoderma is distributed widely in the east Qinghai-Tibetan plateau.

It should be emphasized that the prevalence in young yaks was high (>94%) compared to adult yaks which was around 50%. The reason for this observation is not clear, but it could be partially explained by difference in the movement constraint used in young and adult yaks. Yak’s milk is an important source of income for Tibetan nomadic people. Thus, young yaks are tethered to stakes so that they cannot move and drink milk freely from their mothers. In contrast, adult yaks were able to move freely and escape from adult warble fly attack by swishing their tails or running away. Therefore, it would be relatively difficult for warble flies to lay their eggs on adult yaks. Also, acquired resistance against Hypoderma infection may play a significant role in the infection in adult yaks [10, 16].

The seasonal development and migration patterns of Hypoderma larvae in yak bodies in the study areas were also quite similar. It has been reported that the 1st instar larvae of H. lineatum and H. sinense parasitized in the esophagus and those of H. bovis in the epideral fat of the spinal canal [2, 4, 15, 21]. However, as observed in our previous study [13], a large proportion of 1st instar larvae were found not only in the esophagus but also in the rumen. Moreover, the larvae were detected in other organs in late summer to early autumn (August and September). Unfortunately, species identification was not conducted in this study. Although morphological identification for the species of 1st instar larvae is impossible [15], DNA identification of species would help to understand the migration patterns of H. bovis, H. lineatum and H. sinense in yaks.

Hypoderma in the study areas showed the similar seasonal larval development, and therefore, ivermectin was administered at the same time (October) to yaks grazing the different sites. The timing of the ivermectin administration was chosen based on our previous research that showed that administration in October resulted in complete elimination of the larvae from yaks, but that administration in September or November resulted in incomplete elimination [13].

Ivermectin has been previously shown to be effective for deworming Hypoderma larvae [1, 7, 11, 14, 18, 20]. However, the continuous use or overuse of ivermectin could be accompanied by side effects to the animals treated [5, 9], environmental pollution [3], induction of drug-resistant flies or impairment of human health as a result of consumption of meat containing the chemical [22]. Therefore, it is ideal to use the minimum effective dose of ivermectin with the minimum number of treatments per year [14]. This study showed that a single administration of full and half doses of ivermectin could completely eliminate the parasites from yaks. A single quarter dose administration was also quite effective and eliminated most of the larvae from yaks. Therefore, we determined that a single dose of 125 µg/kg bw of ivermectin was an optimal dose for treatment.

As expected, a large-scale control trial conducted in this study was successful. Although complete elimination of Hypoderma larvae from treated yaks was not achieved and a small number of Hypoderma lumps remained in some yaks after treatment, the trial showed a high cost-performance ratio. We believe that the following factors may also have contributed to the successful control. Before implementation of this project, a training program for the Tibetan local nomads was conducted to teach the method and the appropriate time of year for administration of ivermectin to yaks. In addition, since restraining yaks is very difficult, administration of the drug by splashing onto the animals seemed to be a suitable method which is also less laborious, easier and safer than injection. In conclusion, the control method used in this study would be suitable for use in the Qinghai-Tibetan plateau to reduce economic losses due to Hypoderma infection and contribute to the improvement of animal health and subsequently human health.

We assumed multiple species of Hypoderma were detected in this study, because we had detected two species of Hypoderma, H. bovis and H. lineatum, in yaks at the same sites of this study in a previous study (unpublished). However, regrettably, we did not identify the species of Hypoderma detected in the present study. Because the difference in the species construction could affect the local epidemiology and larval migration pattern, species identification by morphological and DNA sequence characteristics should be incorporated into further studies.

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